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European Journal of Pharmacology 414 (2001) 1–7

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Review

A common genetic variant of luteinizing hormone; relation to normal and aberrant pituitary–gonadal function

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Received 6 November 2000; received in revised form 23 November 2000; accepted 28 November 2000

Abstract

Mutations of the luteinizing hormone (LH) subunit genes are extremely rare. Only one polymorphic LHβ gene variant makes an exception. In 1992, an immunologically anomalous form of LH was found in a healthy woman, and it was subsequently found to be caused by two point mutations leading to two amino acid substitutions in the LHβ subunit. Of the two point mutations, $Trp^{8}Arg$ and $Ile^{15}Thr$, the first one is mainly responsible for the altered immunoreactivity and the latter one introduces an extra glycosylation site into Asn^{13} of the mutated LHβ peptide. The frequency of this variant LHβ allele differs widely between ethnic groups, being most common in aboriginal Australians (carrier frequency $>50\%$; allelic frequency 28.3%) and totally lacking from Kotas of Southern India. Functional differences have been detected when wild-type LH and variant LH have been compared. Variant LH possesses increased in vitro bioactivity, whereas its half-life in circulation is shorter in comparison to wild-type LH. Also the regulation of the variant LHβ gene differs due to additional changes in its promoter sequence. Correlations of occurrence of variant LH with various clinical conditions involving LH function suggest that it represents a biologically less active form of LH and may be related to borderline suppression of gonadal function, including subfertility. In this article, we will review the current information about the differences observed in structure and functions between the wild-type and variant LH, as well as their possible pathophysiological correlations.

1. Introduction

Normal development and function of the reproductive system are dependent on complex hormonal interactions. The two pituitary gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), play an essential role in regulating the mammalian gonadal function. Together with human chorionic gonadotrophin (hCG) and thyroid-stimulating hormone (TSH), they belong to the family of glycoprotein hormones. The similar signal transduction mechanism employed by LH, FSH, hCG and TSH is based on shared subunit structure among the hormones. All of the hormones harbor a heterodimeric structure, consisting of noncovalently associated common α subunit and a unique β subunit, specific for each of the hormones (Pierce and Parsons, 1981) (Fig. 1A). The individual subunits have no known biological activity, and only heterodimerization leads to hormonal function. The interaction with hormone-specific receptor and signal transduction are determined by the β subunit (Gharib et al., 1990).

Glycoprotein hormones act by binding to the extracellular domain of their specific receptors, belonging to the G-protein associated seven transmembrane domain receptors with a long extracellular extension (Tena-Sempere and Huhtaniemi, 1999) (Fig. 1B). After ligand binding, the transmembrane part of the receptor is coupled to G-protein, transducing the normal signal mainly by activating adenylate cyclase, but also other signalling systems are activated by gonadotrophins, including inositol phosphate turnover, calcium and chloride fluxes, and mitogen-activated protein (MAP) kinase mediated pathways. Several protein phosphorylation reactions occur in the next steps and finally they lead to activation of steroidogenesis and other target cell responses, including growth and differentiation. FSH and TSH have their cognate specific

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)00756-7

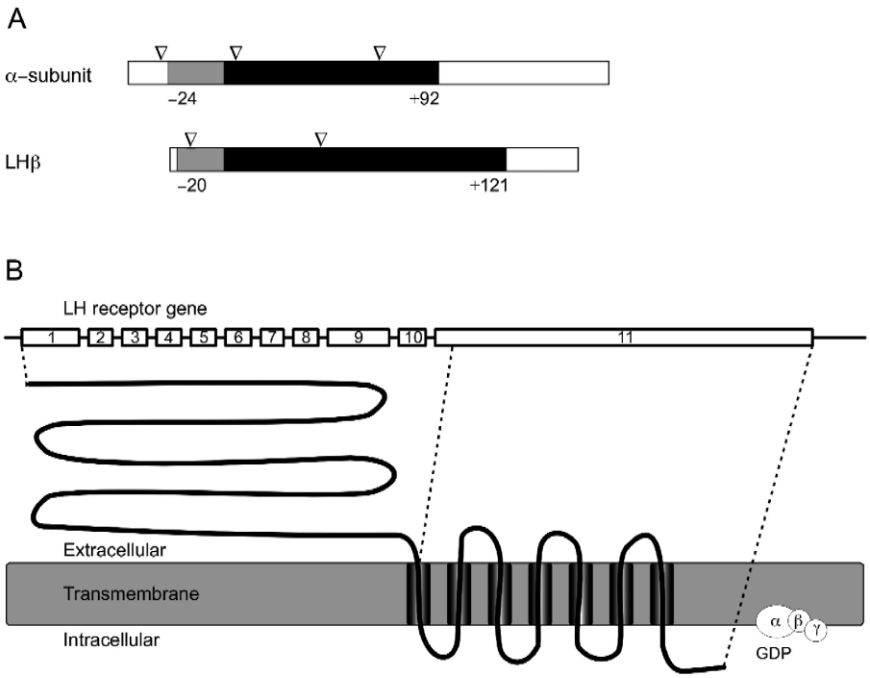

Fig. 1. (A) Schematic presentation of the human LH subunits. The black areas show the protein coding regions, and grey areas the signal peptides. Arrows above the bars indicate the relative positions of the *N*-linked carbohydrate side-chains. (B) Schematic structure of the LH receptor gene and protein. The receptor has a large extracellular domain in NH_2-terminus, and seven transmembrane segments with short intracellular tail in exon 11.

receptors, but LH and hCG bind to the same luteinizing hormone receptor (Huhtaniemi and Catt, 1981).

2. Human mutations of LH subunit genes

Mutations in gonadotrophin and gonadotrophin receptor genes are very rare (Themmen and Huhtaniemi, 2000). Selection is apparently effective due to the direct effects of such mutations on fertility and reproduction. However, few sporadic mutations and some common LH variants have been reported (Table 1). The studies of the variants have been elucidating with respect to the complex actions of LH.

2.1. Common α subunit

As mentioned above, the same α subunit is shared by all four glycoprotein hormones. Thus, a mutation in α

Table 1
List of currently known mutations and polymorphisms in human LHβ gene

Locus	Type	Nucleotide change	Amino acid change	Male phenotype	Female phenotype	Alteration in:		Reference
						Bioactivity	Immunoreactivity	
Exon 3	missense	$CA^{221}G \rightarrow CGG$	$Gln^{54} \rightarrow Arg$	absence of spontaneous puberty		absent	normal	Weiss et al. (1992)
Exon 2	missense	$T^{82}GG \rightarrow CGG$	$Trp^{8} \rightarrow Arg$	delayed tempo of pubertal progression	slightly surpressed fertility	increased in vitro decreased $T_{1/2}$ in circulation	poorly detected by α/β specific antibodies	Pettersson et al. (1994); Furui et al. (1994); Rajkhowa et al. (1995); Haavisto et al. (1995); Raivio et al. (1996); Takahashi et al. (1998, 1999)
		$AT^{104}C \rightarrow ACC$	$Ile^{15} \rightarrow Thr$					
Exon 3	missense	$G^{364}GT \rightarrow AGT$	$Gly^{102} \rightarrow Ser$	infertility	menstrual disorders, infertility	decreased in vitro	normal	Liao et al. (1998); Ramanujam et al. (1999, 2000)
Exon 3	missense	$G^{52}CA \rightarrow ACA$	$Ala^{-3} \rightarrow Thr$			normal	normal	Jiang et al. (submitted)

The nucleotide number was counted according to the translation start site (Fiddes and Talmadge, 1984); the intronic sequences are excluded. From Jiang et al., with permission.

subunit would have widespread effects. An individual with a common α subunit mutation would be both hypothyroid and hypogonadal, and pseudohermaphrodite if a genetic male (Huhtaniemi, 2000). Moreover, pregnancy would probably be seriously compromised because of lack of hCG. Only one amino acid substitution has been found in this subunit. A substitution of Glu^{56} by Ala^{56} was discovered in an ectopically secreted form of hCG α subunit in a patient with undifferentiated carcinoma of the femoral region (Nishimura et al., 1986); the mutation was apparently somatic. It was hypothesized that the mutation in question causes an extension of the preceding hydrophobic segment, which may lead to aggregation of the altered α subunit, alteration in glycosylation, and altered tertiary structure or self-dimerization, causing failure to associate with the β subunit (Nishimura et al., 1986). Because of the expected profound effects, it is unlikely that germ-line mutations in the α subunit gene will be discovered.

2.2. LHβ subunit

Altogether, four genetic variants have been found in the β subunit of LH, and only one has a profound effect on activity of the synthesized hormone. This case was described in 1992 in a family with several infertile males (Weiss et al., 1992). The proband was a 17-year-old man with delayed puberty. His serum LH level was twofold elevated, FSH level was within the normal range, but testosterone was low. Since exogenous hCG stimulated increase in serum testosterone level, primary gonadal failure could be excluded as a cause for the biased hormone levels. Although his LH was immunologically active, in vitro bioassay demonstrated that it was totally devoid of bioactivity. His family history with three infertile maternal uncles and consanguinity in the pedigree referred to an inherited defect, most likely in the structure of LHβ gene. All the female relatives studied were normal. A homozygous A-to-G missense mutation was found in codon 54 of the LHβ gene, causing a Gln to Arg substitution. Functional studies confirmed that the inactivating effect of the hormone was due to the single amino acid replacement. When the mutated gene was co-transfected with wild-type α subunit to Chinese hamster ovary cells, immunoreactive LH α/β-heterodimer was expressed, but the hormone was unable to bind to the LH receptor. Since testicular testosterone production is crucially dependent on LH stimulated testosterone production, this finding explains the subject's phenotype: lack of pubertal development and arrested spermatogenesis. His normal male-type sexual differentiation until birth can be explained by normal action of the placental gonadotrophin, hCG, in utero (Huhtaniemi, 1994). This is the tropic stimulus of fetal testicular testosterone production, which induces male sexual differentiation before birth. The role of pituitary LH becomes important only after birth.

Two variants of the LHβ subunit seem to be population-specific according to screenings of various ethnic groups. The first one of these was found upon single strand conformational polymorphism (SSCP) analysis of 103 individuals in Singapore. A G-to-A transition in nucleotide 1502 replaces Ser^{102} with Gly in exon 3 of the LHβ subunit gene (Roy et al., 1996). The amino acid replacement causes a conformational change that might affect the normal function of LH. Interestingly, this mutation has been found in two Singaporean women with infertility and endometriosis (2/52, 4%) (Liao et al., 1998) and in five Chinese men with infertility (5/170, 2.9%) (Ramanujam et al., 1999). Recently, the Ser^{102}Gly mutation has been screened in Finnish, Danish, Bengali and Rwandan populations, but no positive sample for the mutation was found (M. Jiang, T. Lamminen, P. Pakarinen, J. Hellman, P. Manna, R. Herrera, I. Huhtaniemi, submitted). Therefore, it seems that the occurrence of this mutation is specific for some Asian populations.

Upon screening for the LHβ gene polymorphisms in various populations, a new variant was found. Direct sequencing revealed a heterozygous $G^{52}A$ mutation in exon 2, resulting in Thr^{-3}Ala amino acid substitution in signal peptide part of the LHβ subunit (M. Jiang, T. Lamminen, P. Pakarinen, J. Hellman, P. Manna, R. Herrera, I. Huhtaniemi, submitted). The variant was found in 3 out of 100 DNA samples from Rwanda, thus indicating African origin of the mutation, and possibly also its limited existence in populations of this continent. Protein sequencing of a recombinant form of the LH variant revealed that the mutation did not interfere with signal peptide cleavage. Neither were the secretory proportions of intact LH, free α or free β significantly different between human embryonic kidney cells expressing wild-type or variant LHβ chain, indicating that the signal peptide mutation does not influence markedly the efficacy of heterodimerization. However, a minor decrease in LH-stimulated cAMP response was found when signal transduction was compared between wild-type and variant LH. The cause for this difference remains unclear, but it could be caused by the slightly altered signal peptide structure that may influence the intracellular processing and tertiary structure of the hormone and thus its interaction with the LH receptor.

2.3. The common LH variant

In studies on testing the suitability of various monoclonal antibodies for LH measurement, a healthy woman with immunologically anomalous form of LH was found (Pettersson et al., 1992). One particular monoclonal antibody, recognizing an epitope present in the intact LH α/β dimer, was unable to detect her LH whereas all the other antibody combinations (α, β and α/β specific) detected normal LH level in her serum. The bioactivity and bioactivity/immunoreactivity ratio of the aberrant form of LH were normal, in accordance to her fertility; she had two

children. Later on, an immunological method for detecting the LHβ status with respect to the variant form of LH was developed (Haavisto et al., 1995; Nilsson et al., 1998). In this method, serum LH is measured by two immunofluorometric assays, one detecting only wild-type LH (assay 1), and the other detecting wild-type and variant LH with a similar stoichiometry (assay 2). According to the ratio of LH by assay 1:assay 2, an individual's LH status can be determined (Fig. 2). Homozygotes for wild-type LHβ have normal ratio (1–2), heterozygotes have low ratio (0.5–0.75) and homozygotes for variant LHβ have a zero ratio (close to 0). The LH levels were measured in the Finnish family using the new method, and the ratios of the two assays showed that the proband and her mother were homozygotes for the variant allele and the father and children were heterozygotes for the variant LH.

Because the FSH and TSH levels of the person with the anomalous LH were normal, it was concluded that there must be a structural alteration in her LHβ subunit gene responsible for the aberrant interaction with the LH dimer-specific monoclonal antibody. Sequencing of the LHβ subunit gene revealed, indeed, two amino acid changing mutations: $Trp^{8}Arg$ ($TGG \rightarrow CGG$), and $Ile^{15}Thr$ ($ATC \rightarrow ACC$) (Pettersson et al., 1994; Nilsson et al., 1998). These same mutations were also found by two Japanese groups in females with infertility and whose LH was undetectable by an immunometric assay kit (Furui et al., 1994; Okuda et al., 1994). The $Ile^{15}Thr$ mutation creates an extra glycosylation signal (Asn-X-Thr) into the LHβ chain, which introduces an oligosaccharide side chain into Asn^{13} (Suganuma et al., 1996). This mutation changes the structure of LH closer to hCG, which has glycosylated Asn^{13} in the same position of the amino acid chain. This extra glycosylation site probably rescues the LHβ subunit from disulfide-linked aggregation or homodimerization (Suzuki et al., 2000). Experiments with recombinant human variant LH proteins with either of the two mutations showed that the altered immunoreactivity was mostly due to the $Trp^{8}Arg$ mutation (Suganuma et al., 1996).

We have studied the frequency of the variant LHβ allele extensively in various ethnic groups, and its distribution shows interesting features (Table 2). First of all, the variant LHβ allele seems to be a universally common polymorphism and thus makes it different from the other LH variants that have so far been found only in specific populations. Secondly, the carrier frequency of variant LH varies from 0% (Kotas of South India) to 53.5% (Aboriginal Australians). The wide variability of frequency may imply that this polymorphism has offered an advantage in certain environments during human evolution (Nilsson et al. 1998; Huhtaniemi, 2000). This is possible because the highest frequencies of the variant LHβ allele are detected in nomadic populations living in unfavorable external conditions, e.g. Aboriginal Australians and Lapps of Northern Scandinavia (Nilsson et al., 1997, 1998). Variant LH may represent an ancient form of common LH because of its wide occurrence in populations with different evolutional origins. Because of its marginally suppressive effect on reproduction (see below), variant LH may be gradually vanishing from the gene pool, but the pace is diverse in different populations. In genetic isolates, like Aboriginal Australians, or Finnish Lapps, the pace is slower due to the enrichment of the variant LH allele and of "genetic drift", i.e. inefficient mixing of genes in isolated populations.

Functional differences have been shown between variant LH and wild-type LH. Findings on serum samples of

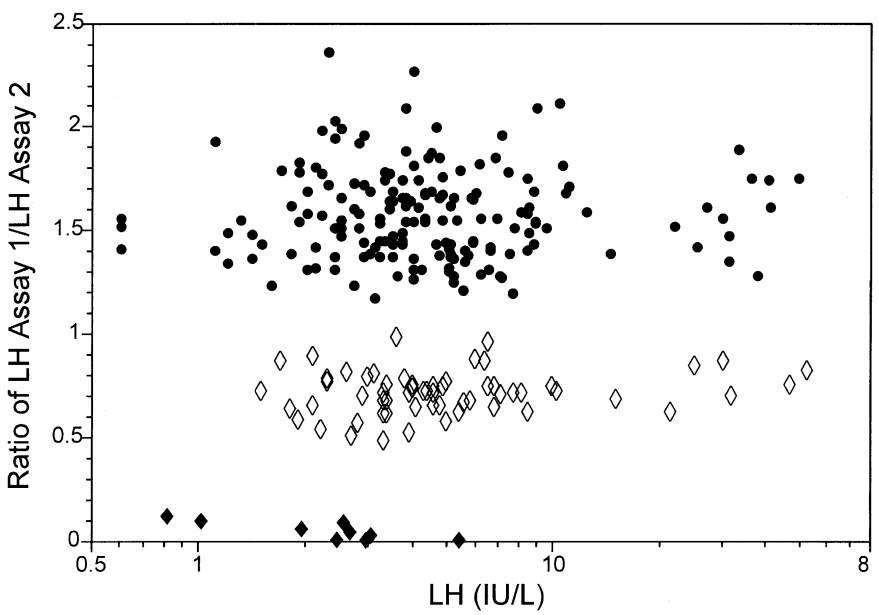

Fig. 2. The distribution of 249 normal Finnish subjects into normal (black dots), low (white squares) and zero (black squares) ratio groups according to results of the ratios of LH measured by assays 1 and 2. From Haavisto et al. (1995) with permission.

Table 2
The carrier frequencies of LHβ variant ($Trp^{8}Arg/Ile^{15}Thr$) in different ethnic groups

Population	Samples (*n*)	Frequency (%)	95% Confidence interval
Finland (Lapps)	129	41.9	33.4–50.4
Finland	249	27.7	22.1–50.34
Estonia	296	21.3	16.6–26.0
Poland	199	21.1	15.3–26.7
Sweden	376	18.9	14.9–22.9
United Kingdom	212	15.1	10.3–19.9
Netherland	63	14.3	5.7–22.0
Italy	294	13.6	9.7–26.4
South Africa (black)	78	17.9	9.4–26.4
China	91	14.3	7.1–21.2
Thailand	244	12.7	8.5–16.9
Japan	258	12.0	8.0–16.0
United States (black)	251	14.7	10.3–19.1
United States (hispanic)	196	7.1	3.5–10.7
Australian Aboriginals	99	53.5	43.7–63.3
Jordan	40	12.5	2.3–22.7
Spain (Vascos)	102	6.9	2.0–11.8
Mexico (Mayans)	40	5.0	0.6–16.9
South India (Kotas)	47	0	0
Iceland	232	22.4	17.0–27.8
Faeroe Islands	190	25.3	19.3–31.3
Greenland	202	21.6	16.0–27.2
Singapore Chinese	191	8.4	4.5–12.33
Singapore Malays	121	12.4	6.5–18.27
Singapore Indians	150	6.0	2.2–9.8
Turkish	30	16.67	9.8–30
Russia	130	11.5	6.0–17.0
Czech	257	17.5	12.8–22.1
Rwanda	100	19	7.4–26.7
Bengali	78	2.56	0–4.4

The data were summarized from Nilsson et al. (1997, 1998), Ramanujam et al. (1999), Elter et al. (1999), Starka et al. (1999) and Jiang et al., unpublished data. From Jiang et al., with permission.

variant homozygotes (Haavisto et al., 1995) and on the recombinant protein (Suganuma et al., 1996) show that variant LH is more active than wild-type LH in bioassays in vitro, but it has shorter half-life in circulation. The effect of the shorter half-life of hormone can possibly be at least partly compensated by enhanced promoter activity of the gene. When promoter sequences of the wild-type and variant LHβ subunit genes were compared, eight additional nucleotide changes were found. Functional testing of the promoters in transfected cell cultures showed that the activity of the variant LHβ promoter was about 40% higher than that of the wild-type LHβ promoter (Jiang et al., 1999).

Since the wild-type and variant LH hormones are functionally different, variant LH having short but more potent action, and that of wild-type LH being more prolonged but less potent, it is expected that the phenotypic expression of the hormone types may be different. Also the combination of the two forms of LH, i.e. heterozygotes for wild-type and variant LH, could differ from both of the homozygous phenotypes. It may also be speculated that heterozygous individuals have the most dramatic phenotypic changes due to combined action of the two differently functioning hormone types. Because of the rarity of variant LH homozygotes, no clear picture of the phenotypic effects of the homozygous variant has emerged. Therefore, more information is available of the much more common variant LH heterozygotes.

On the basis of fertility of the first Finnish women homozygous for the variant LHβ allele, the variant LH does not seem to have major effects on fertility. Neither did a later study from Finland show any association of variant LH with recurrent spontaneous abortions (Tulppala et al., 1998). Notably, however, the variant LH allele was more common in relatively overweight women than normal weight women with recurrent spontaneous abortions. On the other hand, the first studies from Japan concerned sporadic cases of variant LH homozygotes with recurrent spontaneous abortions, menstrual irregularities, infertility and polycystic ovarian syndrome (PCOS) (Furui et al., 1994; Okuda et al., 1994; Suganuma et al., 1995). In addition, heterozygocity for the variant has been associated with infertility problems in the Japanese population (Takahashi et al., 1998, 1999) and in a predominately Jewish population from Boston (MA, USA) (Cramer et al., 2000).

Of some interest also are the findings on connections of variant LH and PCOS patients from different populations. In the United Kingdom, the frequency of heterozygous variant LH was higher in obese PCOS patients than in either obese controls or in non-obese PCOS patients (Rajkhowa et al., 1995). The presence of the variant allele did not change the clinical or hormonal expression of the disorder in women with PCOS. Completely opposite results were obtained in a study of PCOS patients from Finland, the Netherlands and the United States. In these populations, the frequency of variant LH was similar in obese and non-obese controls, as well as in non-obese PCOS subjects, but 5–7-fold lower in obese PCOS subjects (Tapanainen et al., 1999). The lower frequency of variant LH in obese PCOS subjects may imply that the variant allele somehow protects obese individuals from developing symptomatic PCOS (Tapanainen et al., 1999). The similar frequency of variant LH in healthy non-obese and obese women, and likewise non-obese PCOS patients, suggests that obesity is not directly related to the variant allele. The differences between populations are interesting: why is the connection seen in three populations, but not in the United Kingdom, despite identical diagnostic criteria? Is it due to the nutritional factors or the type of obesity? In these studies, about 2/3 of the British PCOS patients were obese, whereas only 1/3 of patients were obese in other populations studied.

When considering which one of the two hormone types, variant LH or wild-type LH, is more potent in vivo, breast cancer can be used as a model disease. In a total of about 539 breast cancer patients from Helsinki, Finland, those

heterozygous for variant LH were at the time of diagnosis on average 4 years older than wild-type LH individuals ($P < 0.01$; P. Sálven, H. Nevanlinna and I. Huhtaniemi, unpublished observation) implying that variant LH is less potent in stimulating ovarian estrogen production, which may be linked to occurrence of breast cancer. However, this finding needs to be verified using a larger number of patients. On the other hand, in a study of older women with breast cancer from New York, diagnosed at an age of 50 or older, no altered risk was observed between carriers of variant LH allele or wild-type LH homozygotes (Akhmedkhanov et al., 2000), suggesting no difference in LH action. In the latter study, however, the patients studied had mixed ethnic origin, which may mean diverse genetic background and additional factors, such as environmental or nutritional influences, affecting the onset of breast cancer. Curiously, the finding that women heterozygous for variant LH have significantly elevated levels of serum estradiol, testosterone and sex hormone-binding globulin compared to wild-type LH women alludes to higher activity of variant LH (Rajkhowa et al., 1995). Likewise, our very recent findings indicate that postmenopausal women with variant LH had significantly higher levels of serum testosterone than wild-type controls (M. Hill, R. Hampl, I. Huhtaniemi, L. Stárka, unpublished). How these findings comply with the overall impression of weaker activity of variant LH remains to be resolved.

Also in the male, phenotypic associations with variant LH have been found. The age of onset of puberty is similar in wild-type LH and heterozygous variant LH boys, but the tempo of its progression is significantly slower in variant LH boys (Raivio et al., 1996). In elderly men, serum LH level increases significantly with age, whereas production of testosterone decreases, probably due to a primary disturbance of Leydig cell function (Vermeulen, 2000). Indicators of frailty, i.e. age-related disability characterized by generalized weakness, impaired mobility and balance, and poor endurance, are related to reduced testosterone level and thus also to higher LH concentration in circulation. Testosterone levels and the degree of frailty were not different in the wild-type LH persons compared with the heterozygotes of variant LH. But again, a positive correlation between variant LH allele and fat mass as well as leptin was observed among these elderly men (van den Beld et al., 1999).

3. Conclusions

The question about the phenotypic effect of the LH variant is intriguing. In larger population scanning studies, the only clear connection between variant LH and pathological function so far found is in PCOS, but also here a contradictory result exists when comparing patients from the United Kingdom to other populations studied. The comparison of results from different populations is problematic due to the complex nature of the diseases studied. Breast cancer, PCOS, recurrent spontaneous abortions, frailty of old age and progression of puberty are all regulated by several genes, and population-specific features, such as genetic background, environmental effects and nutrition, contribute to expression of these conditions. Interestingly, in several studies, obesity seems to be related to LH action. In women with relative obesity, variant LH seems to function as a protective factor against symptomatic PCOS. Similarly, the variant LH allele is more common in elderly men with increased fat mass and higher leptin concentrations. In this respect, our recent findings that LH overexpressing transgenic mice are obese provides an interesting experimental model for future studies (J. Kero, J. Nilson, R. Keri, I. Huhtaniemi, unpublished). Novel experiments should be performed to cast further light on possible links between obesity and LH, and more generally to the role of variant LH.

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