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Alterations in gonadal steroidogenesis in individuals expressing a common genetic variant of luteinizing hormone ☆

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Abstract

Some pathologies of the pituitary-gonadal function 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, by elucidating some less well characterized facets 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) β-subunit gene, where two point mutations cause two alterations in the amino acid sequence ($Trp^{8} \rightarrow Arg$ and $Ile^{15} \rightarrow Thr$) and introduce an extra glycosylation signal to Asn^{13}. The carriers of this variant gene are largely healthy, but certain mild differences in their gonadal function have been found, as reflected by alterations in gonadal steroidogenesis, pubertal development and predisposition to diseases such as infertility, polycystic ovarian syndrome, and breast and prostatic cancer. The purpose of this chapter is to review the current knowledge of the occurrence, special functional features and clinical correlates of this LH variant.

Keywords: Luteinizing hormone; Polymorphism; Puberty; Aging; Prostatic cancer; Breast cancer; Polycystic ovarian syndrome (PCOS)

1. Introduction

Recent studies on the molecular pathogenesis of disturbances of pituitary-gonadal function have revealed rare conditions caused by mutations in the genes of gonadotropins and their receptors (for recent reviews, see [1–6]). These conditions are extremely rare, but very informative as regards the role LH and FSH in the regulation of ontogeny and mature functions of the reproductive organs. The few cases of mutations in the FSHβ and LHβ genes invariably inactivate the hormonal function, whereas in those of the cognate receptors, both activating and inactivating mutations have been described. The inactivating gonadotropin receptor mutations cause in both sexes various disturbances of sexual maturation, and infertility or subfertility. The activating LH receptor mutations cause in boys gonadotropin-independent precocious puberty (testotoxicosis), and the one activating FSH receptor mutation so far detected presented with maintenance of spermatogenesis in the absence of circulating FSH [3]. Female phenotypes with the activating LH and FSH receptor mutations have not yet been described.

Besides clear-cut mutations with a distinct pathological phenotype, multiple polymorphic variants with no, or mild phenotypic expression have been, or are likely to be discovered, in all genes. With the LH β-subunit gene, four genetic variants, besides the wild-type form, are currently known (Table 1). One of them, a point mutation, results in an LH form with persistent immunoreactivity but total lack of bioactivity. The affected individual, a phenotypic male, presented with lack of spontaneous pubertal development [7]. Two other mutations cause one [12] or two [8–10] amino acid changes in the LH β-chain, and infor-

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Table 1
The currently known genetic variants of the LH β-subunit gene

Mutation(s)	Amino acid change(s)	Phenotype	Reference
160A → G	$Gln^{54} \rightarrow Arg$	Male with lack of spontaneous puberty	[7]
22 T → C	$Trp^{8} \rightarrow Arg$	Variable mild alterations in gonadal function	[8–10]
44 T → C	$Ile^{15} \rightarrow Thr$		
304G → A	$Ser^{102} \rightarrow Gly$	Infertility (?)	[11,12]
6 silent polymorphisms in exons and introns		None	[11]

mation is currently accumulating to indicate that mild alterations in gonadotropin action are found in individuals homo- or heterozygous for these polymorphisms. We have studied extensively the latter $Trp^{8} \rightarrow Arg/Ile^{15}Thr$ LHβ mutation, and the present chapter summarizes the key findings.

2. A common genetic variant of LH

We discovered the Variant (V) of LH upon testing the reactivity of various monoclonal antibodies (Mab) against LH in two-site immunofluorometric Delfia® (Wallac OY, Turku, Finland) assays [13,14]. No LH could be detected in the serum of an apparently healthy woman with one of the antibody combinations, using a Mab recognizing an antigenic epitope present only in the intact LH α/β dimer. Since the FSH and TSH levels of the subject were normal, as well as LH measured by the other Mab combinations and by an in vitro bioassay, it was concluded that her LH β-subunit must be structurally aberrant. The LHβ gene of the subject was sequenced, and two missense point mutations were discovered [9,10]. Subsequent studies have demonstrated that the V-LH gene is a common genetic variant with wide variation in frequency in different ethnic groups (Table 2). Concomitantly with our studies the same mutations were described in reports from Japan [8,17,18]. Although the sequence variability of the LH β-subunit was already detected in early biochemical studies [19], only the recent molecular biological studies have revealed its genetic variant nature.

2.1. Alterations in gene and protein structure of V-LH

The two point mutations in the V-LHβ gene change amino acid tryptophan in position 8 to arginine and that in position 15 from isoleucine to threonine. In addition, the latter mutation introduces an extra glycosy-

Table 2
Carrier frequency of the variant LHβ gene in various populations [10,15,16]

Population/geographic area	No. of samples	Carrier frequency (%)	95% confidence interval
Finland (Lapps)	129	41.9	33.4–50.4
Finland	249	27.7	22.1–33.3
Sweden	376	18.9	14.9–22.9
Estonia	296	21.3	16.6–26.0
Iceland	232	22.4	17.0–27.8
Faroe Islands	190	25.3	19.3–31.3
Greenland	204	21.6	16.0–27.2
Poland	199	21.1	15.3–26.7
United Kingdom	212	15.1	10.3–19.9
The Netherlands	63	14.3	5.7–22.9
Russia	130	21.5	15.2–27.8
Italy	294	13.6	9.7–17.5
Vascos (Spain)	102	6.9	2.0–11.8
Jordan	40	12.5	2.3–22.7
Kotas (South India)	47	0	0
Thailand	244	12.7	8.5–16.9
China	91	14.3	7.1–21.2
Japan	258	12.0	8.0–16.0
South Africa (Black)	78	17.9	9.4–26.4
United States (Black)	251	14.7	10.3–19.1
United States (Hispanic)	196	7.1	3.5–10.7
Mayans (Mexico)	40	5.0	0.6–16.9
Australia (Aboriginal)	99	53.5	43.7–63.3

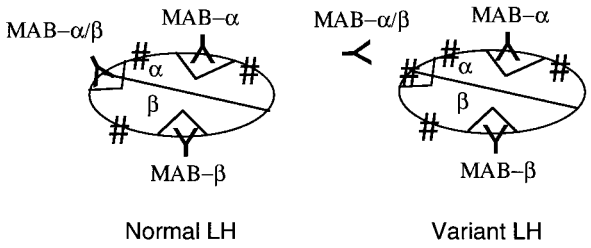

Fig. 1. Schematic presentation of the reason for aberrant immunoreactivity in individuals with the variant form of LH in two-site immunometric assays. Either the changes in the amino acid sequence and/or an extra carbohydrate chain in the LH-chain block or eliminate the antigenic epitope present in the α/β LH dimer. Combination of Monoclonal AntiBody (MAB) α/β and MAB-β was used in Assay 1, and MAB-α and MAB-β in Assay 2. ♯♯ = carbohydrate side chain.

lation signal (Asn-X-Ser/Thr) to asparagine in position 13. The same sequence is preset and glycosylated in the hCG β-subunit and LH β-subunit of other animal species, and the studies on recombinant V-LH by Suganuma et al. [20] suggest that this site is glycosylated in V-LH as well, thus increasing the number of carbohydrate side chains in the LH molecule from 3 to 4. The amino acid changes themselves and/or the extra carbohydrate side chain alter the α/β dimer-specific antigenic epitope of the LH molecule in such a way that it is not recognized by some α/β conformational Mabs. On the basis of this restricted recognition of V-LH by such Mabs, it is possible to set up an immunological detection method for the LH variant status by using two immunoassays with different Mab combinations (Fig. 1). One assay (assay 1) uses the α/β specific Mab and a subunit-specific Mab, and it only detects the Wild-Type (WT) LH molecules. The other assay (assay 2) uses two subunit-specific antibodies and detects equally both LH forms. When the ratio of LH measured by assay 1/assay 2 is calculated, the ratio is in theory 1 with individuals homozygous for WT-LH, about 0.5 in heterozygotes, and close to 0 in homozygotes for V-LHβ. In this way, by using two immunoassays, the LH variant status of an individual can be detected from the serum sample in a reliable and simple fashion. We have subsequently developed an allele-specific DNA hybridization assay for detection of the V-LHβ gene, which is useful for screening of large numbers of DNA samples [10].

2.2. Structure-function relations

The intrinsic in vitro bioactivity of V-LH is on average 30% higher than that of WT-LH, as reflected by its significantly higher ratio of bioactivity/immunoreactivity (i.e. bio/immuno ratio) [15]. In contrast, the bioactivity in vivo is apparently lower due to a faster half-time ($T_{1/2}$) of V-LH in circulation as compared to WT-LH [15,20]. Suganuma et al. [20] have prepared in CHO cells recombinant forms of V-LH containing either one or both of the two amino acid alterations of V-LH, and their findings suggest that the altered behavior of V-LH is predominately due to the mutation in codon 8. The putative extra carbohydrate side chain in Asn^{13} is therefore apparently of lesser importance for the restricted immunoreactivity. However, since CHO cells are unable to add terminal sulfate groups to the carbohydrate side chains, it still remains to be studied how V-LH with sulfated carbohydrate termini, akin to LH of pituitary origin, would behave at the receptor site and in circulation.

Liver has a specific receptor binding sulfated glycoproteins, thereby shortening their circulatory $T_{1/2}$ [21]. This explains the faster elimination of LH compared with structurally similar FSH, the carbohydrates of which are not sulfated. Thus, V-LH, apparently possessing 4 terminally sulfated carbohydrate side chains, instead of 3 in WT-LH, is expected to display shorter $T_{1/2}$ in circulation. How the higher in vitro bioactivity, but shorter $T_{1/2}$ in circulation, is reflected in the overall action of the hormone in vivo, i.e. whether it is more or less active than WT-LH, remains to be clarified. If the latter is the case, then V-LH synthesis should be more active than that of WT-LH, in order to maintain balance in the pituitary–gonadal feedback regulation. Indeed, this may be the case since we have observed several additional point mutations in the promoter of the V-LHβ gene, and in transfection experiments the V-LHβ promoter seems to be more active than that of the WT-LH [22].

2.3. Physiological and pathophysiological correlates

We have numerous observations indicating small but significant differences in the ovarian and testicular function of individuals homo- or heterozygous for V-LHβ. Some findings suggest stronger action of V-LH, whereas others suggest weaker stimulation of gonadal function, and at the moment the total picture of the difference in actions of the two LH forms remains unclear.

Healthy women heterozygous for V-LHβ have slightly but significantly elevated circulating levels of estradol, testosterone and Sex Hormone-Binding Globulin (SHBG) in the follicular phase of the menstrual cycle [23], suggesting subtle higher V-LH activity at the ovarian level. Similar stronger action of V-LH is suggested by the less frequently suppressed testosterone levels in aged men carrying the V-LHβ gene [I. Huhtaniemi and K. Pettersson, unpublished observation].

Findings suggesting weaker action of V-LH include the slow progression of puberty in boys heterozygous for V-LH [24]. Likewise, we have preliminarily observed that in breast cancer patients, the age at diag-

nosis is 10 years higher in the women heterozygous for V-LH [I. Huhtaniemi, P. Salven and K. Pettersson, unpublished observation].

Some intriguing findings have been made on V-LH in connection with gynecological disorders. The data from Japan suggest that V-LH might be associated with menstrual disorders [20]. In our studies on women with recurrent spontaneous abortion, V-LH was as prevalent as in the normal population. In contrast, the obese subgroup of these women (body mass index >27 kg/m^2) had a significantly higher proportion of V-LH than non-obese women, 30 vs 60% [25]. Healthy obese women display a normal proportion of V-LH, whereas in women with PolyCystic Ovarian Syndrome (PCOS), the obese subgroup seems to present with lower proportion of V-LH [26]. Hence, V-LH is significantly more prevalent in obese women with recurrent spontaneous abortions, but the opposite is found in obese women with PCOS. We do not yet understand the pathogenetic mechanisms of these disorders, and it also remains obscure how obesity and V-LH are related.

3. Conclusions and future perspectives

To summarize, we have recently detected a common genetic variant of LH. The two point mutations occurring in the LHβ gene introduce two amino acid changes and an extra glycosylation signal to the V-LH β-subunit. These changes alter the immunoreactivity of the V-LH molecule, information which is important for clinicians, because some commonly used LH assays use Mabs not recognizing V-LH, thus causing misinterpretation of LH measurements. The biological activity of V-LH at the receptor site is increased, but its $T_{1/2}$ in circulation is decreased. Whether these differences increase or decrease the overall LH activity in vivo in subjects expressing the V-LHβ gene is not yet totally clear. However, there are already sufficient data to state that the LH-dependent gonadal functions in carriers of the variant gene are slightly but significantly different from WT-LH individuals. The physiological and pathophysiological consequences of V-LH are currently under detailed investigation in our laboratory. Such studies include in vitro and experimental in vivo studies with a recombinant preparation of V-LH. The clinical data so far obtained on delayed puberty, PCOS and hormone-dependent cancer suggest that V-LH plays a predisposing or protecting role in the pathogenesis of various disorders of gonadal function.

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