GENETIC VARIANT OF LUTEINIZING HORMONE IN CZECH REPUBLIC

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Objective. To evaluate the frequency of luteinizing hormone (LH) variant in males and females in West region of the Czech Republic. This species of mutated LH concerns borderline alterations in pituitary-gonadal function, including higher risk for the development of more aggressive forms of prostate carcinoma in males.

Methods. The examined normal population consisted of randomly selected 82 males and 175 females (age range of 14 to 72 years). Variant LH was determined by immunofluorimetric method using two pairs of monoclonal antibodies, one of which detecting both wild-type (w+) and variant LH, while the other detecting only w+-LH.

Results. The carrier frequency of the variant LH allele in the population sample was 17.5 % (12.2 % in males and 20.6 % in females) which was within the range of the European prevalence.

Conclusion. The prevalence of the common variant of LH in the investigated region of West Bohemia was close to that of other Middle-European and North-European populations.

Key words: LH variant – Prevalence in Czech Republic – Immunofluorimetric estimation

The common variant of LHβ subunit was recently discovered by Huhtaniemi and coworkers (Huhtaniemi 1998; Huhtaniemi et al. 1996; Nilsson et al. 1996; Pettersson et al. 1992). This variant occurs worldwide with frequency from 0 to over 50 % in various populations. In most of the Caucasian population, the carrier frequency is about 15 % (Nilsson et al. 1998). The lowest frequency was observed in Kotas ethnics of South India (0 %), the highest in Finnish Lapps (41,9 %) and in Australian Aborigines (51.5 %). The variant allele of the LHβ subunit gene differs from the wild type LHβ subunit gene by two point mutations, both altering the aminoacid sequence (Trp8Arg and Ile15Thr). The latter mutation also introduces an extra glycosylation signal to the LHβ chain. In addition, several mutations are found in the promoter of the variant gene, which is transcriptionally only slightly, while biologically much more active than the wild type gene (Jiang et al., unpublished).

The bioactivity of the LH variant is increased (by *in vitro* assay), but some monoclonal antibodies detecting the intact LHα/β dimer do not recognise it in radioimmunoassay. The circulatory half time of the variant LH is shorter than that of wild type hormone. Although the LH variant has not appeared to be clearly related to gonadal dysfunction, in various clinical conditions it was observed that individual homo- or heterozygotes carrying the variant show several borderline alterations in pituitary-gonadal function such as the significantly delayed puberty in boys carrying the variant LHβ allele (Huhtaniemi 1998; Huhtaniemi et al. 1996). Likewise, in women with polycystic ovary syndrome the frequency of LH variant was found significantly lower in the obese subgroup compared to the lean subjects and healthy controls (Tapanainen et al.

1999). In men, the carriers of the LHβ variant allele maintain higher testosterone levels at advanced age, and may develop the more aggressive forms of prostate cancer (Huhtaniemi et al., unpublished observation).

In conclusion, the variant LH may explain a part of the large inter-individual variation in pituitary-gonadal function, and may be a contributing factor in various disorders of gonadal steroidogenesis. For this reason it seems to be the point of interest in which frequency the variant LH occurs in the normal Czech population.

Subjects and Methods

Subjects. Males and females of age range from 14 to 72 years were randomly selected from the population register in Cheb district in West Bohemia. The examination of the population was a part of iodine deficiency field survey and the subjects were examined mainly with respect to their thyroid status, including physical, laboratory and ultrasound examination. The population studies consisted of 175 females (average age 42, median 45 years, and range 14-72 years) and 82 males (average age 43, median 44 and range 18-65 years). Blood was withdrawn from the cubital vein and serum was frozen until processed in the laboratory.

The protocol of the study was approved by the Ethical Committee, Institute of Endocrinology, Prague.

Immunofluorometric assays of serum LH. For LH determination the method of Pettersson et al. (1992) was used. Th commercial kit Delfia LH spec (Wallac OY, Turku, Finland) which uses two LH β-subunit specific monoclonal antibodies (mAb) served as the reference method assay (assay 2). In the other assay (assay 1) the capture mAb recognises a conformational epitope present in the intact α/β LH dimer but not in the subunits, and the detection mAb recognises an epitope in the subunit α. The procedures followed the instructions indicated by the producer. 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), C. zero ratio (<0.15). The individual with a normal ratio individual has two wild-type LH alleles, while a low ratio individual is heterozygous for the LH variant allele and a zero ratio individual is homozygous for the variant LHβ gene, as confirmed by DNA analysis (Nilsson et al. 1997).

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 and above the lowest standard concentration (0.6 mIU/ml of the WHO International Reference Preparation 80/552).

Statistical evaluation. Because of the strongly skewed distribution in LH and LH-variant and non-linear relations between the age and the levels of LH or LH-variant, the correlations between variables were evaluated by robust non-parametric Spearman's rank correlation test. Only for the comparison of two assay variants the Pearson's correlation after logarithmic transformation of both LH and LH-variant was used. The differences in LH or LH-variant levels between the clusters Age-LH in female wild-type homozygotes and between the clusters Age-LH-variant in female wild-type-LH variant heterozygotes were evaluated by ANOVA followed by Scheffe's test. The values of LH or LH-variant were transformed by power transformation (lambda=0.4) prior to testing because of the skewed distribution of the original data.

Results

The frequency of hetero- and homozygotes and the wild-type LH in males and females, the homogeneity of the population sample as concerns LH secretion and the age dependence were studied.

The overall characterisation of the groups is given in Table 1.

The validity of the discrimination between wild-type LH and heterozygotes is demonstrated in Fig. 1 which represents the correlation between the log plot of LH, determined by assay 1 and 2. There is no overlap between the 95 % confidence interval for predictions of the linear fits in wild-type homozygotes and wild-type-LH-variant heterozygotes.

The frequency of LH-variant in males was 12.2 % (10 heterozygotes versus 72 "wild type" homozygotes) and 20.6 % in females (1 LH-variant homozygote, 35 heterozygotes versus 139 "wild type" homozygotes). From the total number of probands 3.9 % were male heterozygotes, and 13.6 % female heterozygotes. The chi-square test did not reveal any

Table 1: Summary statistics of the data in the population group investigated

"wild type"								
Statistics	**Female**				**Male**			
	Age [years]	**LH_{intact} [mIU/l]**	**LH_{spec} [mIU/l]**	**LH_{nt} / LH_{spec}**	**Age [years]**	**LH_{intact} [mIU/l]**	**LH_{spec} [mIU/l]**	**LH_{nt} / LH_{spec}**
Count	139	139	139	139	72	72	72	72
Average	42.09	23.70	15.78	1.48	43.43	6.41	4.41	1.45
Median	45.00	13.10	9.20	1.49	45.00	5.57	3.88	1.45
Geom. mean	39.84	13.26	8.96	1.48	40.93	5.91	4.07	1.45
SD	12.86	23.62	15.49	0.12	13.61	2.98	2.02	0.06
Minimum	14.00	0.24	0.19	1.19	18.00	1.84	1.28	1.36
Maximum	72.00	114.00	71.80	2.59	65.00	18.40	13.20	1.74
Lower quartile	31.00	5.84	3.99	1.44	34.50	4.42	3.13	1.40
Upper quartile	52.00	37.40	24.20	1.52	53.50	7.30	5.00	1.49
Stnd. skewness	-1.32	6.32	6.05	24.38	-1.28	7.38	7.79	4.50
Stnd. kurtosis	-2.09	3.06	2.34	113.11	-1.40	9.30	11.00	7.89
heterozygotes								
Statistics	**Female***				**Male**			
	Age [years]	**LH_{intact} [mIU/l]**	**LH_{spec} [mIU/l]**	**LH_{nt} / LH_{spec}**	**Age [years]**	**LH_{intact} [mIU/l]**	**LH_{spec} [mIU/l]**	**LH_{nt} / LH_{spec}**
Count	36	36	36	36	10	10	10	10
Average	41.47	10.92	16.34	0.70	40.80	4.11	5.65	0.73
Median	43.50	4.71	7.05	0.72	42.00	3.68	4.86	0.73
Geom. mean	39.37	5.38	9.05	0.60	39.01	3.49	4.79	0.73
SD	12.38	11.95	17.23	0.13	11.91	2.80	3.94	0.03
Minimum	17.00	0.03	1.33	0.00	21.00	1.75	2.35	0.68
Maximum	62.00	41.40	59.90	0.83	59.00	10.90	15.30	0.78
Lower quartile	35.50	2.34	3.29	0.70	34.00	2.00	2.92	0.71
Upper quartile	51.00	18.65	26.55	0.74	48.00	4.01	5.38	0.75
Stnd. skewness	-0.93	2.98	2.77	-12.32	-0.51	2.41	2.51	-0.14
Stnd. kurtosis	-0.97	0.27	-0.04	34.74	-0.24	2.34	2.52	-0.51

** LH-variant homozygote included*

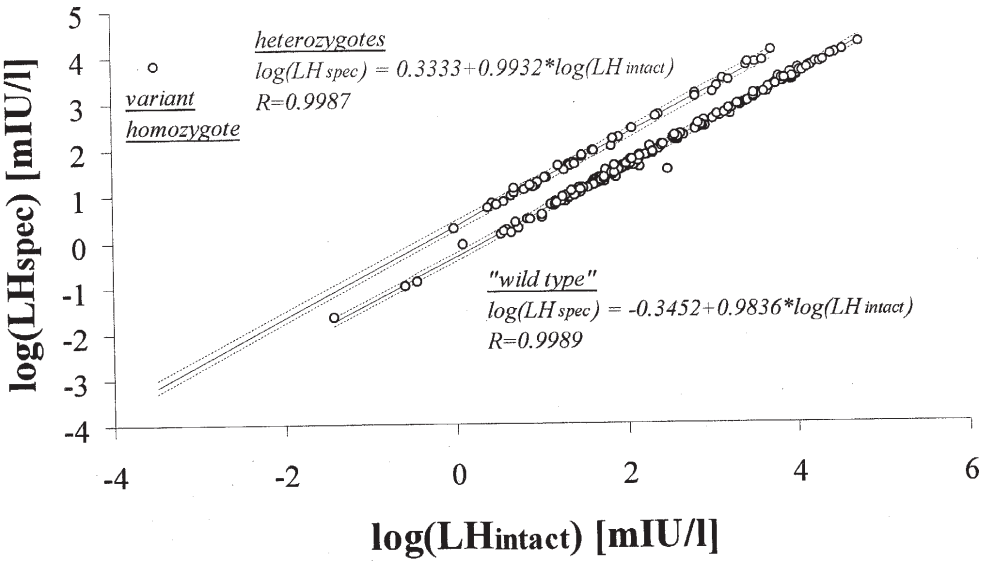

Fig. 1 Determination of LH by two assay variants. Pearson's correlations between log(LH_{intact}) and log(LH_{spec}) in wild-type and in heterozygotes.

significant differences in the frequency between males and females.

The LH age dependence in male wild-type homozygotes showed a slight but significant positive correlation (r=0.3303, n=72, P<0.0054). The correlation coefficient in LH-variant age dependence in male heterozygotes was even higher, but unsignificant because of the low number of measurements (r=0.5228, n=10, P<0.1168). Both in female wild-type homozygotes and heterozygotes a pronounced positive age dependence was found in LH (r=0.6668, n=139, P<0.0001) and LH-variant (r=0.5825, n=36, P<0.0006), respectively. The increased levels of LH and LH-variant in perimenopausal and postmenopausal women caused the pronounced age dependence of both LH and LH-variant. The certain variance found in LH and LH-variant levels was caused by the presence of samples obtained from women regardless to the phase of menstrual cycle.

The cluster analysis of LHspec and LHintact showed no clustering of LH or LH-variant neither in wild-type male homozygotes nor in male heterozygotes when plotted with age. However, in females the well separable clusters appeared in both types of LH.

LH vs. Age cluster plot in female wild-type homozygotes showed four groups. The first group involved females in fertile age, the second group constituted two outliers in fertile age, and the third and the fourth groups involved perimenopausal and postmenopausal women (Fig. 2). Significant differences between the clusters in the levels of LH were found using ANOVA followed by Scheffe's test (Fig. 3).

LH-variant vs. Age cluster plot in female wild-type-LH-variant heterozygotes showed four groups. First and the second group involved females in fertile age, the third group was LH-variant homozygote in fertile age and the fourth group involved perimenopausal and postmenopausal women (Fig. 4). Significant differences between clusters in the levels of LH-variant were found using ANOVA followed by Scheffé's test (Fig. 5).

Discussion

The survey of worldwide frequency of the LH variant in various populations revealed that it is a universally common polymorphism (Nilsson et al. 1997). With the exception of Australian Aborigines, the highest frequencies of variant LHβ allele, in general, are in the northern parts of Eurasia, while the frequency decreases with increasing geographic distance and is lowest in Asian or in Asia originating (native Mexican Indians) populations. It has been suggested that the structure of variant LH is closer to hCG than that of wild-type LH, especially in the first 20 N-terminal amino acids. The glycosylation signal presented by amino acids 13-15 is

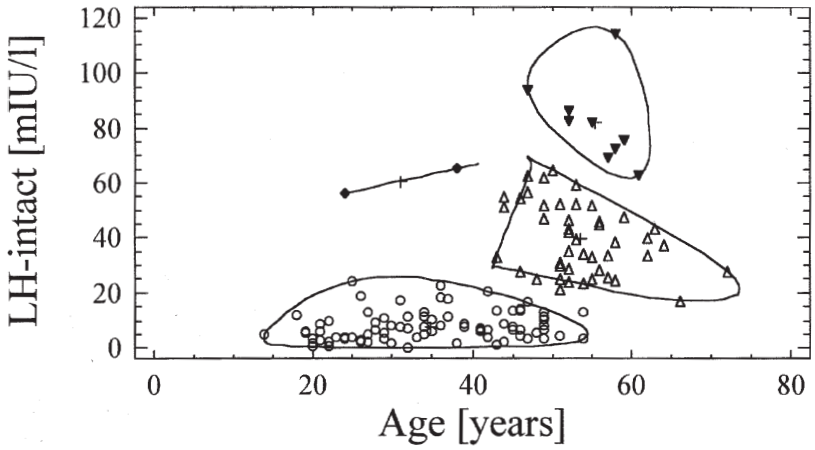

Fig. 2 Clustering of the LH levels when plotted against age in female wild-type homozygotes. The method of group average combined with the euclidean between-cluster distance was used. Cluster 1 – circles, cluster 2 – rhombuses, cluster 3 – triangles, cluster 4 – reversed triangles, centroids – crosses.

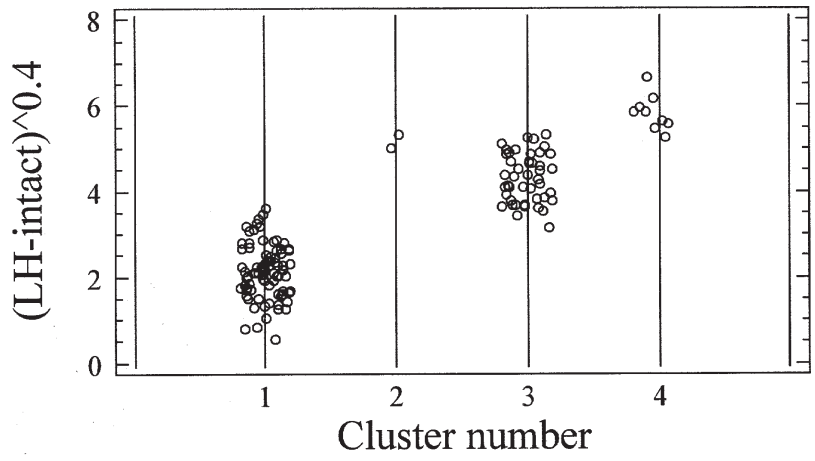

Fig. 3 Differences in the levels of LH between the clustered groups in wild-type female homozygotes. For the statistical evaluation ANOVA followed by Scheffe's test was used. The significant differences (P<0.05) were found between clusters 1-2, 1-3, 1-4, 3-4.

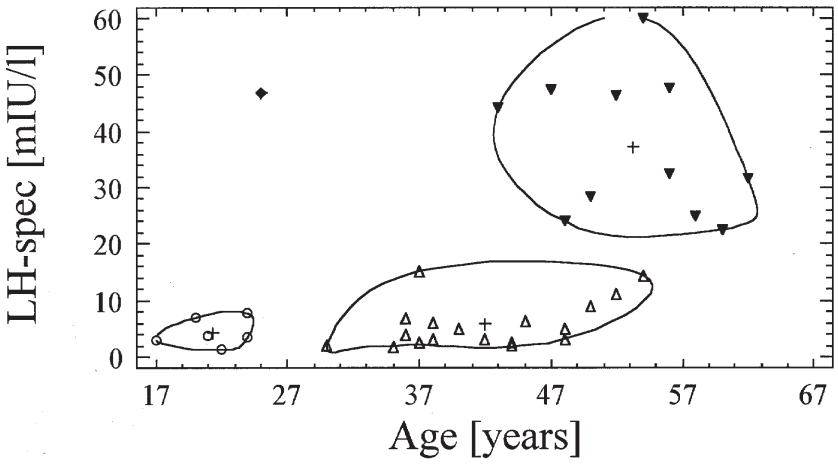

Fig. 4 Clustering of the LH-variant when plotted against age in female "wild type"-LH-variant heterozygotes. The method of group average combined with the euclidean between-cluster distance was used. Cluster 1 – circles, cluster 2 – rhombuses, cluster 3 – triangles, cluster 4 – reversed triangles, centroids – crosses.

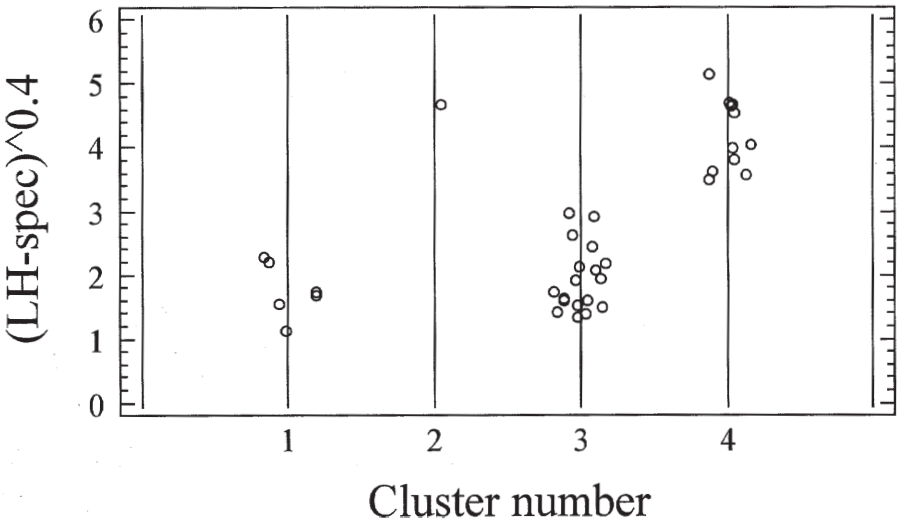

Fig. 5 Differences in the levels of LH-variant between the clustered groups in female "wild type"-LH-variant heterozygotes. For the statistical evaluation ANOVA followed by Scheffe's test was used. The significant differences (P<0.05) were found between clusters 1-3, 1-4, 2-3, 3-4.

identical for hCGβ and variant LHβ, not for wild-type LHβ. It seems that the variant LH represents an evolutionary older form of LH. Occurrence of both types indicates that the process of natural selection has not yet been accomplished. It is either because the evolutionary divergence is too recent or the functional advantage of the wild-type LH is only marginal. From this point of view it is not surprising that the prevalence in Czech Republic does not differ from that in the North or Central Europe.

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References

Nilsson C, Jiang M. Pettersson K, Iitiae A, Maekelae M, Simonsen H, Easteal S, Herrera R, Huhtaniemi IT: Determination of a common genetic variant of luteinizing hormone using DNA hybridization and immunoassays. Clin Endocrinol (Oxford) **49**, 369-376, 1998

Nilsson Ch, Petterson K, Millar RP, Coerver KA, Matzuk M, Huhtaniemi IT: Worldwide frequency of a common genetic variant of luteinizing hormone: an international collaborative research. Fertil Steril **67**, 998-1004, 1997

Huhtaniemi IT: Alterations in gonadal steroidogenesis in individuals expressing a common genetic variant of luteinizing hormone. Proc. Xth ICSH, Quebec City, June 1998. S-40

Huhtaniemi I, Pakarinen P, Nilsson Ch, Pettersson K, Tapanainen J, Aittomäki K: Polymorphism and mutations of gonadotropin and gonadotropin receptor genes. In: The Ovary: Regulation, Dysfunction and Treatment (Eds. M Flicori, C Flamigni), Elsevier, Amsterdam 1996

Pettersson K, Ding Y-Q, Huhtaniemi I: An immunologically anomalous luteinizing hormone in healthy woman. J Clin Endocrinol Metab **74**, 164-171, 1992

Tapanainen JS, Koivunen R, Fauser BMJ, Taylor AE, Clayton RN, White D, Franks S, Antilla L, Pettersson K, Huhtaniemi IT: A new contributing factor to polycystic ovarian syndrome – the genetic variant form of luteinizing hormone. J Clin Endocrinol Metab **84**, 1711-1715, 1999

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