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Molecular classification of pituitary adenomas — in search of criteria useful for high-throughput studies

Klasyfikacja molekularna gruczolaków przysadki — w poszukiwaniu kryteriów przydatnych do badań wysokoprzepustowych

Jadwiga Żebracka-Gala[1], Adam Rudnik[2], Kornelia Hasse-Lazar[1], Dawid Larysz[3], Michał Jarząb[4], Jolanta Krajewska[1], Piotr Bażowski[2], Barbara Jarząb[1]

[1]Department of Nuclear Medicine and Endocrine Oncology, MSC Cancer Centre and Institute of Oncology, Gliwice Branch, Poland
[2]Department of Neurosurgery, Silesian University School of Medicine, Poland
[3]Radiotherapy Department, MSC Cancer Centre and Institute of Oncology, Gliwice Branch, Poland
[4]III Department of Radiotherapy and Chemotherapy, MSC Cancer Center and Institute of Oncology, Gliwice Branch, Poland

Abstract

Introduction: The mechanism of pathogenesis of pituitary adenomas is still unknown, and it shows differences in pituitary cells of different origin.

The aim of our study was to analyse the gene expression profile of pituitary hormones and their precursor genes: *PRL*, *GH*, *POMC*, *TSHb*, *LHb*, *FSHb*, and *CGA* by QPCR in particular types of pituitary adenomas, and to evaluate the results in the context of sample selection for microarray studies.

Material and methods: Analysis of the gene expression profile was performed in 84 samples of pituitary adenomas, by real-time quantitative PCR (QPCR).

Results: As expected, expression of *GH* gene was significantly higher in somatotropinomas than in prolactinomas ($p<0.05$). For *POMC* gene we noticed lower expression in all pituitary adenomas, except adrenocorticotropinomas ($p<0.05$). In the case of *PRL* gene, the highest expression was observed; PRL+ adenomas were in third place. *LHb* and *FSHb* genes showed the highest expression, respectively, in LH-producing and FSH-producing pituitary adenomas; however, our analysis did not show statistically significant differences between LH-producing and FSH-producing adenomas**.**

Conclusions: Our study showed that *GH* is a characteristic gene for somatotropinomas. We drew a similar conclusion for *POMC* gene and adrenocorticotropinomas.

However, the results that we obtained for *PRL*, *TSHb*, *LHb*, *FSHb*, and *CGA* genes indicate that evaluation of gene expression is not sufficient for classification of particular subtypes of pituitary adenomas. **(Endokrynol Pol 2016; 67 (2): 148–156)**

Key words: *pituitary adenoma; gene expression; QPCR*

Streszczenie

Wstęp: Mechanizm odpowiedzialny za patogenezę gruczolaków przysadki nie został jeszcze w pełni wyjaśniony i wykazuje różnice w różnych typach komórek przysadki.

Celem badania była analiza profilu ekspresji genów kodujących hormony przysadkowe i ich prekursory: *PRL*, *GH*, *POMC*, *TSHb*, *LHb*, *FSHb, CGA* w poszczególnych typach gruczolaków przysadki oraz ocena uzyskanych wyników w kontekście wyboru próbek do badań mikromacierzowych.

Materiał i metody: Analizę ekspresji genów przeprowadzono za pomocą ilościowej reakcji PCR w czasie rzeczywistym (QPCR) na materiale 84 gruczolaków przysadki.

Wyniki: Ekspresja genu *GH* była znamiennie wyższa w gruczolakach somatotropinowych (GH+) w porównaniu z prolaktynowymi (PRL+). Zaobserwowano również wzrost ekspresji tego genu w guzach GH+ w stosunku do gruczolaków immunohistochemicznych ujemnych. Dla genu *POMC* wykazano niską ekspresję we wszystkich badanych grupach gruczolaków, z wyjątkiem gruczolaków kortykotropinowych (ACTH+). Najwyższą ekspresję genu *PRL* zaobserwowano w gruczolakach somatotropinowych; gruczolaki prolaktynowe były na trzecim miejscu. Dla genów *LHb* i *FSHb* nie zaobserwowano statystycznie znamiennych różnic pomiędzy gruczolakami LH+ i FSH+.

Wnioski: W niniejszym badaniu potwierdzono, że gen *GH* jest charakterystyczny dla gruczolaków somatotropinowych, podobnie jak gen *POMC* dla gruczolaków kortykotropinowych. Jednakże, wyniki uzyskane dla genów *PRL*, *TSHb*, *LHb, FSHb* i *CGA* wskazują, że ocena ekspresji genów nie jest wystarczająca dla prawidłowej klasyfikacji poszczególnych podtypów gruczolaków przysadki.
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Słowa kluczowe: *gruczolak przysadki; ekspresja genu; QPCR*

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Jadwiga Żebracka-Gala M.D., Department of Nuclear Medicine and Endocrine Oncology; MSC Cancer Centre and Institute of Oncology, Gliwice Branch, Poland, Wybrzeże AK 15, 44–100 Gliwice, phone: +48 32 278 94 52, e-mail: jzebracka@io.gliwice.pl

Introduction

Pituitary tumours are relatively frequent intracranial tumors and constitute a very interesting model: they share the properties of well-differentiated endocrine tumors, which are able to secrete hormones, characteristic for the cell of origin and the ability for invasive growth, characteristic for neoplastic cells [1]. Among pituitary adenomas there is a wide variety of phenotypes: from hormone-producing tumours to non-functioning adenomas. Most functioning pituitary adenomas secrete prolactin (~40%) [2–4], about 20% secrete growth hormone [5], ~10% secrete corticotropin [1, 3], ~10–15 % secrete glycoprotein hormones (LH, FSH) [1], and less than 1% secrete thyrotropin [6]. The other pituitary adenomas (~5%) are negative for hormone secretion, and these are referred to as immunohistochemically negative (null adenomas) [7]. Pituitary tumours have been classified in different ways. The oldest classification was based on cellular characteristics using haematoxylin and eosin stains on resected tissues; however, this classification did not take into account clinical symptoms or hormone production by the adenoma. With the advent of immunohistochemical tests, tumours are now classified according to the characteristic hormone staining and electron microscopic changes. This classification is in general agreement with the reported clinical signs and symptoms. Currently, this is the main way to distinguish different types of pituitary tumours. However, it is expected that in the future, molecular and genetic techniques will also be applied. At the present time, microarray studies are a powerful method for global analysis of gene expression profile, opening up new horizons in molecular systems. In the case of pituitary adenomas there is still little specific knowledge about the gene expression profile differentiating particular subtypes of pituitary tumours.

In our study we focused on one of the crucial steps of microarray studies: sample selection. For this purpose, we used real-time quantitative PCR (QPCR), which is the most common method for fast, accurate, sensitive, and cost-effective gene expression analysis in many samples concurrently. It generates high-quality data without the requirement of additional validation, and it is applied to validate data obtained by higher throughput technologies such as microarray.

The main goal of our study was to analyse the gene expression profile of pituitary hormones and their precursor genes: *PRL*, *GH*, *POMC*, *TSHb*, *LHb*, *FSHb* and *CGA* by QPCR in particular types of pituitary adenomas, and to evaluate the results in the context of sample selection for microarray studies.

Material and methods

The study was approved by the Ethics Committee of Maria Sklodowska-Curie MSC Cancer Centre and Institute of Oncology in Gliwice.

Patients

The studied group of consisted of 84 patients operated on for pituitary adenoma with application of endoscopic transnasal transsphenoidal approach (ETTA) in the Department of Neurosurgery of the Medical University of Silesia, Poland. There were 41 women and 43 men. The mean age was 53.2 years (18–70 years) for the female subgroup and 52.7 years (20–77 years) for men. In 10 cases, tumours had increased features of invasiveness, i.e. the presence of tumour cells in samples of dura obtained during the surgery, in histopathological examination. Moreover, all aforementioned tumours infiltrated lateral walls of the sella, so they were classified as III or IV degree in Knosp's Classification of Cavernous Sinus Invasion. In six cases surgical resection was performed as a reoperation. In all of the aforementioned six cases surgery was the first reoperation.

Tumours

The analysis of gene expression was performed in 84 samples of pituitary adenomas, collected with the cooperation of the Department of Neurosurgery of the Silesian Medical University in Katowice. Fragments of pituitary adenomas were taken intraoperatively and stored in RNA at 4°C. All adenomas were histopathologically verified. As judged by postoperative immunohistochemistry (IHC), there were 13 GH+, 26 PRL+, 8 ACTH+, 5TSH+, 11 LH+, 5 FSH+ and 16 null adenomas (with negative immunohistochemistry toward the previous hormones). The percentage of TSH-positive adenomas in the study group was relatively high compared to published data; however, we collected so many tumours intentionally because we wanted to analyse the gene expression level also in rare functional pituitary adenomas. Moreover, using a smaller group of TSH+ pituitary adenomas in statistical analysis might have led to biased results in this analysis.

The GH+, PRL+, ACTH+ and TSH+ adenomas were grouped as functional adenomas (FA) due to the clinical symptoms caused, while LH+, FSH+, and null adenomas were described clinically as non-functioning tumours (NFA).

Isolation of RNA

Total RNA was extracted from homogenised frozen tissue using Mini Kits (Qiagen GmbH, Hilden, Germany).

Table I. *Amplicons used for quantitative real-time PCR measurement of analysed genes*
Tabela I. *Sekwencje starterów i sondy do ilościowej reakcji PCR dla genów badanych oraz genów kontrolnych*

Gene	Name	Gene ID	Primer F sequence	Primer R sequence	Probe	Probe sequence
Investigated genes						
PRL	Prolactin	NM_000948.2	AAAGGATCGCCATGGAAAG	GCACAGGAGCAGGTTTGAC	18	TCCTGCTG
GH	Growth hormone	NM_000515.3	CCAACAGGGAGGAAACACAA	GACACTCCTGAGGAACTGCAC	19	GGCTGGAG
POMC	Proopiomelanocortin	NM_000939.2	CAGGAGAGCTCGGCAAGTAT	GGCTCTTCTTCCCCTCCTT	82	CTCCTCTG
TSHb	Thyroid-stimulating hormone, beta subunit	S70587.1	CAGCACAATGGATACGCATAA	CAGCACAATGGATACGCATAA	33	TCCCAGCTC
FSHb	Follicle-stimulating hormone, beta polypeptide	NM_000510.2	TGGTGTGCTGGCTACTGCT	CCTTGAAGGTACATGTTTTCTGG	20	CTGGCTGG
LHb	Luteinizing hormone, beta polypeptide	NM_000894.2	GCTACTGCCCCACCATGA	GCTACTGCCCCACCATGA	71	CTGGCTGC
CGA	Glycoprotein hormones, alpha polypeptide	NM_000735.2	TCTCCATTCCGCTCCTGAT	GGGAGAAGAATGGGTTTTCC	61	TTGCCCAG
Reference genes						
ACTB	β-actin	NM_001101.2	ATTGGCAATGAGCGGTTC	GGATGCCACAGGACTCCAT	11	CTTCCAGC
ATP6V1E1	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E1	NM_001696.2	AAGCCGGCTGGATCTCAT	GCATTTGCACCAAACAAGG	3	CCCAGCAG
B2M	β-2-microglobulin	NM_004048.2	TTCTGGCCTGGAGGCTATC	TCAGGAAATTTGACTTTCCATTC	42	CATCCAGC
EIF3S10	Eukaryotic translation initiation factor 3, subunit 10 theta	NM_003750.1	AGTAGAGCGCCTGTACCATGA	GCGTGTATTGGAGGCAGAAT	61	TTGCCCAG
GUSB	β-glucuronidase	NM_000181.1	CGCCCTGCCTATCTGTATTC	TCCCCACAGGGAGTGTGTAG	57	CTGGGGCC
UBE2D2	Ubiquitin-conjugating enzyme E2D 2	NM_003339.2	AATGGCAGCATTTGTCTTGA	CACAACAGAGAACAGATGGACAA	67	CTCCAGCA

RNA quantity was measured by NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA) minispectrophotometer, and the quality was estimated by Agilent 2100 using RNA 6000 Nano Assay (Agilent Technologies, Santa Clara, CA, USA). RNA integrity, assessed by RIN index, was within the 4.5–9.1 range.

cDNA synthesis

cDNA was synthesised from 500 ng of total RNA by Omniscript Kit (Qiagen GmbH, Hilden, Germany), with a mixture of oligo-dT and random nonamer primers (Sigma-Aldrich, ST. Louise, USA) and 10U RNAse inhibitor (Fermentas Thermo Fisher Scientific, Walthman, USA). The reaction was carried out at 37ºC for one hour.

Quantitative real-time reverse transcription-PCR (QPCR)

Analysis of gene expression was performed by real-time quantitative PCR with the use of fluorescent probes from the Universal Probe Library (Roche, Basel, Switzerland). Amplicons were designed using a web-based application (www.roche-applied-science.com/sis/rtpcr/upl) (Table I). QPCR was carried out in a 96-well optical reaction plate using an ABI Prism 7700 Sequence Detection System (Applied Biosystems). Five microlitres of template cDNA (equivalent to 500 ng of total RNA) were added to 15 μl of PCR reaction mix containing 10 μl TaqMan Universal PCR Master Mix (Life Technologies, Carlsbad, CA, USA), 1 μl forward and reverse primers (200 nM), 1 μl probe (100 nM), and water. Thermal cycling condition were as follows: 50°C for two minutes (incubation and activation AmpErase UNG), 95°C for 10 minutes (activation AmpliTaq Polymerase DNA), 95°C for 15 seconds (denaturation), and 60°C for one minute (annealing and extension). Every sample was examined in duplicate. The standard curve, used in experiments, was prepared from serial dilutions of human reference RNA (Stratagene, La Jolla, CA, USA). Expression of the examined genes was normalised to the reference index, obtained by calculation of the geometric mean of the reference genes expression: *ACTB*, *EIF3S10*, *ATP6V1E*, *UBE2D2*, *B2M*, and *GUS B.*

Statistical analysis

geNorm application software for Microsoft Excel was used to identify the most stable reference gene under the described conditions, and to determine the optimal number of reference genes required for reliable normalisation of QPCR data.

To determine between-group differences, we used Mann-Whitney nonparametric test. Differences were considered significant at $p < 0.05$. The fold change was calculated by dividing medians of expression.

Results

The results of the analysis for each gene: *PRL*, *GH*, *POMC*, *TSHb*, *LHb*, *FSHb* and *CGA* were presented in the form of a report showing the raw data: Ct value and the relative of the template, which was read from the standard curve. The expression of the investigated genes was normalised with relation to a reference index, obtained by calculation of the geometric mean of expression of reference genes: *ACTB*, *EIF3S10*, *ATP6V1E*, *UBE2D2*, *B2M*, and *GUS B*. The comparison was performed in particular types of pituitary adenomas: FA/ /null, PRL+/GH+, PRL+/null, GH+/null, GH+/ACTH+, ACTH+/null, LH+/FSH+, LH+/null and FSH+/null. We observed significant differences in gene expression in particular types of pituitary adenomas (Table II).

PRL gene

The highest expression of *PRL* gene was observed in pituitary adenomas, which were GH+ in the IHC study; PRL+ adenomas were in the third place. We observed also that expression of *PRL* gene was the lowest in FSH+ adenomas. When we compared all functional adenomas (FA) with immunohistochemically-negative adenomas (null) *PRL* gene showed 3.5x higher expression in FA; however, the difference was not significant.

GH gene

In a comparison of GH+ and other groups of functional adenomas we observed significantly higher expression of *GH* gene in the GH+/PRL+ comparison (3674× ↑; $p = 0.005$) (Fig. 1A). Expression of *GH* gene was also significantly higher when we compared immunohistochemically-negative adenomas (null) with GH+ adenomas (23618× ↑; $p = 0.002$) (Fig. 1B) and PRL+ adenomas (6× ↑; $p = 0.015$) (Fig. 1C).

POMC *gene*

The expression of *POMC* gene was similarly low in all pituitary groups except adenomas, which were ACTH+ in the IHC study. Significant differences were observed in the comparison of ACTH+ and null adenomas (32× ↑; $p = 0.01$) (Fig. 2).

TSHb gene

For TSHb gene we observed higher expression in TSH+ adenomas than PRL+, GH+, and FSH+. What is interesting is that we observed also an increase in TSHb gene expression in LH+ adenomas, and the difference between TSH+ and LH+ was not significant. Analysis of particular groups of adenomas showed significant differences only for comparison of TSH+/null (3× ↑; $p = 0.04$) (Fig. 3A) and LH+/null (3.25× ↑; $p = 0.038$) (Fig. 3B).

LHb gene

The highest expression of *LHb* gene was observed in LH+ adenomas, and in second place were PRL+ adenomas. However, our analysis did not show significant differences between LH+ and FSH+ adenomas.

FSHb gene

In the comparison of all groups of pituitary adenomas the highest expression of *FSHb* gene was noticed in FSH+ adenomas. The difference of expression level was significant only for comparison of FSH+/null (28× ↑; $p = 0.006$) (Fig. 4).

CGA gene

In the case of *CGA* gene the highest expression was observed in LH+ adenomas, while the lowest was in GH+ adenomas. Expression of CGA gene was on a similar level in TSH+ and FSH+ adenomas. We did not observe significant differences in comparisons of: FSH+ and LH+ adenomas, FSH+ and TSH+ adenomas, and TSH+ and LH+ adenomas. Gene *CGA* was significantly overexpressed in LH+ adenomas in comparison to null adenomas (13× ↑; $p = 0.0008$) (Fig. 5).

Discussion

One of the first results of a microarray study in pituitary adenomas was published by Evans et al. in 2001 [8]. In recent years researchers have used this high-throughput technique to compare gene expression between normal tissues and pituitary adenomas, and identified many genes associated with particular tumour types [9–15].

Our study was intended to evaluate gene expression of pituitary hormone and their precursor genes: *PRL*, *GH*, *POMC*, *TSHb*, *FSHb*, *LHb*, and *CGA* in particular types of functional and non-functional pituitary adenomas using QPCR technique.

In most cases we obtained results that were consistent with the secretory status of the adenoma subtype. However, comparison of our results with other published studies is rather difficult because of the methods we used. Generally, pituitary adenomas are classified in immunohistochemical tests [16, 17], and molecular techniques are not used. We found confirmation of our results in a studies by Evans at al. [8] and Morris et al. [9], in which gene expression profile was analysed by microarray in different types of pituitary adenomas

Table II. *The expression of investigated genes in different types of pituitary adenomas*

Tabela II. *Wartości względne odzwierciedlające ekspresję genów badanych w poszczególnych typach gruczolaków przysadki*

Type of pituitary adenomas	Number	Gene expression			Descriptive statistic		Comparison of each class to null (p level is given if significant)
		Median	Quartile 1	Quartile 3	Skewness	Kurtosis	
PRL							
GH	13	0.427	0.005	6.012	3.479	12.317	ns
PRL	26	0.273	0.015	55.374	1.883	3.158	ns
LH	11	0.393	0.024	0.899	1.262	0.933	ns
FSH	5	0.088	0.001	1.802	0.548	–2.886	ns
FA*	52	0.125	0.012	6.252	2.837	8.421	ns
NFA*	31	0.087	0.005	0.611	4.436	21.963	
null	15	0.037	0.005	0.611	3.823	14.72	
GH							
GH	13	165.332	0.226	216.951	1.667	2.778	0.002
PRL	26	0.045	0.012	0.376	3.145	9.623	0.015
LH	11	0.018	0.005	0.381	2.82	8.264	ns
FSH	5	0.749	0.001	0.749	–0.073	–2.826	ns
FA*	52	0.176	0.012	41.389	2.81	8.078	0.001
NFA*	32	0.021	0.002	0.475	3.822	16.585	
null	16	0.007	0.001	0.475	3.256	11.255	
POMC							
ACTH	8	0.097	0.062	10.658	1.145	–0.631	0.01
GH	13	0.001	0.0004	0.018	2.113	4.924	ns
PRL	26	0.002	0.001	0.013	5.068	25.776	ns
LH	11	0.002	0.001	0.014	3.262	10.724	ns
FSH	5	0.003	0.003	0.009	0.607	–2.697	ns
FA*	52	0.009	0.001	0.034	4.351	18.675	ns
NFA*	32	0.003	0.001	0.01	2.943	8.471	
null	16	0.003	0	0.01	2.222	4.234	
TSH B							
TSH	5	1.584	0.798	5.456	0.616	–2.595	0.04
GH	13	0.786	0.103	1.522	0.474	–1.336	ns
PRL	14	0.599	0.135	1.447	2.581	6.441	ns
LH	11	1.716	0.475	3.201	1.793	2.212	0.038
FSH	5	1.482	1.006	1.776	–0.635	0.535	ns
FA*	50	0.759	0.128	1.569	2.719	7.693	ns
NFA*	32	0.786	0.411	1.66	3.432	11.964	
null	16	0.528	0.245	1.66	1.18	2.188	
LH B							
GH	12	0.007	0.002	1.371	2.456	6.170	ns
PRL	22	0.264	0.01	0.915	2.569	7.109	ns
LH	11	0.422	0.007	2.213	2.074	4.856	ns
FSH	5	0.03	0.03	3.815	0.278	–1.749	ns
FA*	46	0.038	0.004	0.64	2.962	8.922	ns
NFA*	32	0.095	0.005	1.77	1.881	2.979	
null	16	0.055	0.004	1.77	3.127	10.536	

→

Table II cd. *The expression of investigated genes in different types of pituitary adenomas*
Tabela II cd. *Wartości względne odzwierciedlające ekspresję genów badanych w poszczególnych typach gruczolaków przysadki*

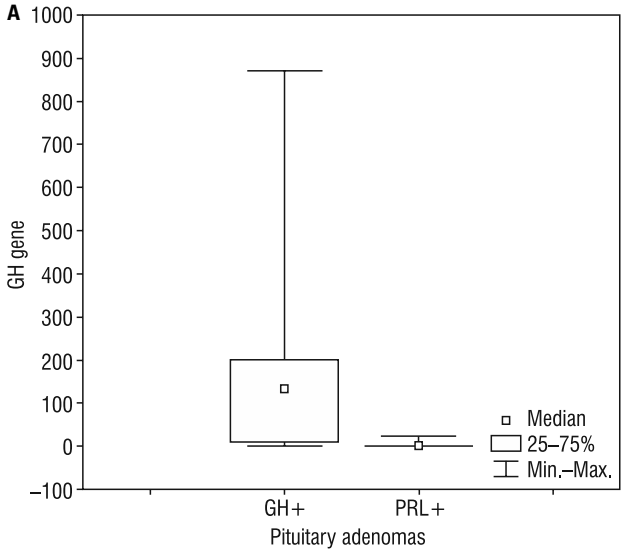
Type of pituitary adenomas	Number	Gene expression			Descriptive statistic		Comparison of each class to null (p level is given if significant)
		Median	Quartile 1	Quartile 3	Skewness	Kurtosis	
FHS B							
GH	12	0.006	0.0002	0.426	2.055	3.151	ns
PRL	20	0.31	0.031	2.915	4.472	20	ns
LH	11	1.11	0.111	3.235	0.524	–1.394	ns
FSH	5	2.525	2.621	7.131	–0.319	–2.959	0,006
FA*	42	0.072	0.001	0.99	6.481	42	ns
NFA*	31	0.305	0.073	3.553	0.931	–0.176	
null	15	0.089	0.013	3.553	1.227	–0.176	
CGA							
GH	16	0.078	0.012	0.646	1.517	0.865	ns
PRL	26	0.227	0.052	1.138	2.776	9.119	ns
TSH	5	0.98	0.008	1.806	0.608	–1.072	ns
LH	11	3.577	0.929	7.219	1.004	–0.139	0,0008
FSH	5	0.88	0.36	0.948	1.938	3.989	ns
FA*	52	0.166	0.029	1.377	2.662	7.961	ns
NFA*	31	0.31	0.18	1.727	2.414	5.44	
null	15	0.271	0.117	1.727	1.366	2.912	

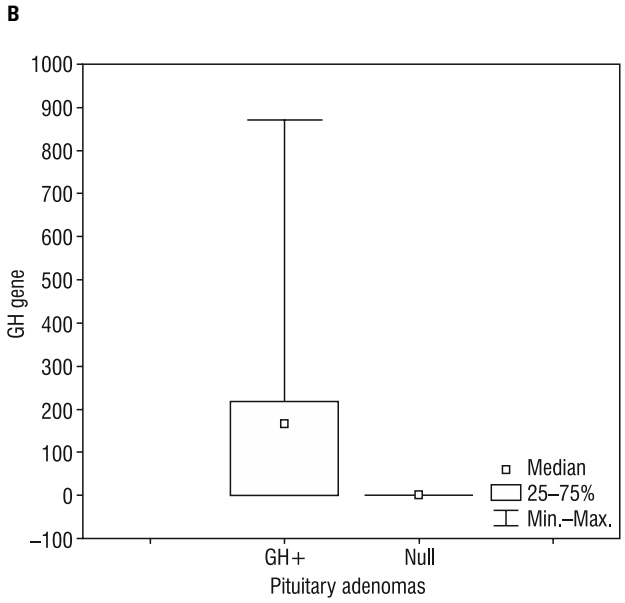
FA — functional adenomas, null— immunohistochemically-negative adenomas, GH — somatotropinomas, PRL — prolactinomas, ACTH — corticotropinomas, TSH — thyrotropic adenomas; LH, FSH — gonadotropic adenomas, NFA — common group of immunohistochemistry negative adenomas and gonadotropic adenomas, FA* — GH+, PRL+, ACTH+, TSH+ adenomas, NFA* — null, LH+, FSH+ adenomas

(normal pituitary, PRL+, GH+, and ACTH+). For *PRL* gene these analyses showed, in comparison to normal pituitary, overexpression in PRL+ adenomas (fold change 2.9×; 3.7× respectively), underexpression in non-functional adenomas (fold change 31.3×; > 10× respectively), ACTH+ adenomas (fold change 10.7×; 2.4× respectively), and in GH+ adenomas (fold change 2.2×; 4.5× respectively). In our study *PRL* gene showed the highest expression in pituitary adenomas that secreted GH, and prolactinomas were in the third place. Our comparison of all functional and immunohistochemically-negative adenomas showed 3.5× higher expression in FA; however, the difference was not significant. The results indicate that *PRL* gene is not a characteristic gene for prolactinomas.

In the case of *GH* gene we observed higher expression in GH+ adenomas in comparison to other groups of pituitary adenomas. According to our expectations, this gene was significantly overexpressed in GH+ adenomas when we compared it with PRL+ (3674×) and also with immunohistochemically-negative adenomas (23618×). Our results are consistent with the results of Evans and Morris, who showed overexpression of *GH* gene in somatotropinomas in comparison to normal pituitary (2× and 9.6×, respectively) and underexpression in prolactinomas (2.2× and 10×, respectively), corticotropinomas (2.8× and > 10×, respectively), and non-functional adenomas (31.3× and > 10×, respectively). Our obtained results confirm that *GH* is a characteristic gene for GH+ adenomas. We drew a similar conclusion for *POMC* gene, expression of which was low in all pituitary adenomas except adrenocorticotropinomas. We observed the same trend in the study of Morris. *POMC* was the most highly expressed gene in ACTH+ adenomas, whereas its expression was lower in GH+ (> 10×), PRL+ (> 10×), and non-functional adenomas (> 10×). Based on the results, we confirm that *POMC* is a characteristic gene for adrenocorticotropinomas.

Another gene which we analysed was *TSHb* encoding *β* subunit of TSH. We showed that its expression was higher in TSH+ adenomas than in PRL+, GH+, and FSH+ adenomas, but we did not observe statistically significant differences between groups of functional adenomas. When we compared particular groups of pituitary adenomas with null adenomas we observed significantly higher expression of *TSHb* gene in TSH+ (3×) and LH+ (3.25×) adenomas. We noticed a similar direction in the above-mentioned studies [8,9]. *TSHb*





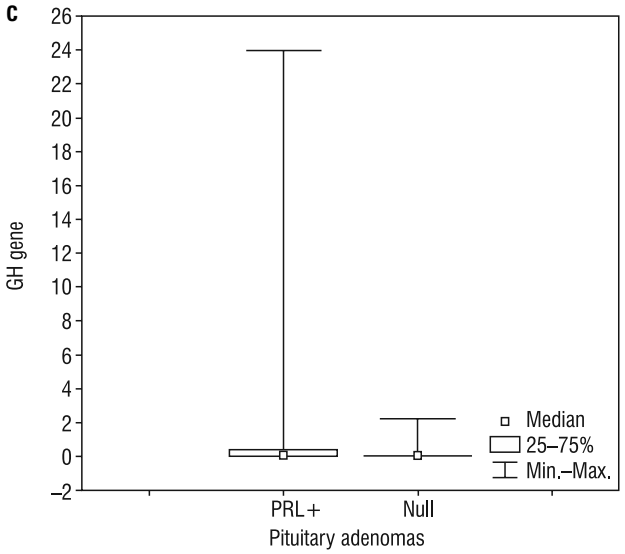


Figure 1. *Comparison of GH expression in:* **A.** *GH+ to PRL+ pituitary adenomas, (U Mann Whitney test, p = 0.005);* **B.** *GH+ to IHC negative (null) pituitary adenomas, (U Mann Whitney test, p = 0.002);* **C.** *PRL+ to IHC negative (null) pituitary adenomas, (U Mann Whitney test, p = 0.015)*

Rycina 1. *Porównanie ekspresji genu GH w:* **A.** *Gruczolakach GH+ w porównaniu z gruczolakami PRL+, (test U Manna Whitneya, p = 0.005);* **B.** *Gruczolakach GH+ w porównaniu z gruczolakami IHC ujemnymi (null) (test U Manna Whitneya, p = 0.002);* **C.** *Gruczolakach PRL+ w porównaniu z gruczolakami IHC ujemnymi (null) (test U Manna Whitneya, p = 0.015)*

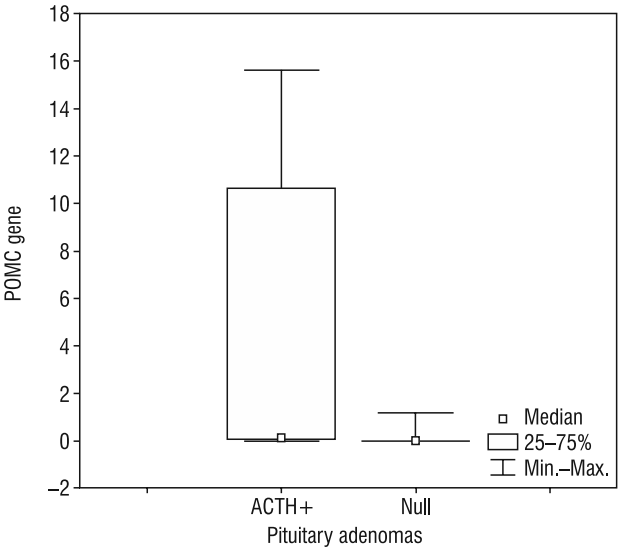


Figure 2. *Comparison of POMC expression in ACTH+ to IHC negative (null) pituitary adenomas, (U Mann Whitney test, p = 0.01)*

Rycina 2. *Porównanie ekspresji genu POMC w gruczolakach ACTH+ w porównaniu z gruczolakami IHC ujemnymi (null), (test U Manna Whitneya, p = 0.01)*

gene was underexpressed in PRL+ (17.2× and > 10×, respectively), GH+ (16.2× and > 10×, respectively), and non-functional adenomas (2.4× and >10×, respectively), in comparison to normal pituitary. The next two genes are expressed in pituitary adenomas that are clinically non-functional, but they can produce glycoprotein hormones: LH or FSH. According to our expectations, expression of *LHb* and *FSHb* genes was the highest, respectively, in LH+ and FSH+ adenomas; we noticed lower expression of these genes in PRL+, GH+, and immunohistochemically-negative adenomas. We found similar results in the studies by Evans et al. and Morris et al. Expression of *LHb* gene was lower in PRL+ (4× and 2×, respectively), GH+ (4.9× and 8×, respectively), and in non-functional adenomas (6.2× and 7.7×, respectively). *FSHb* gene was underexpressed in PRL+ (10.2×

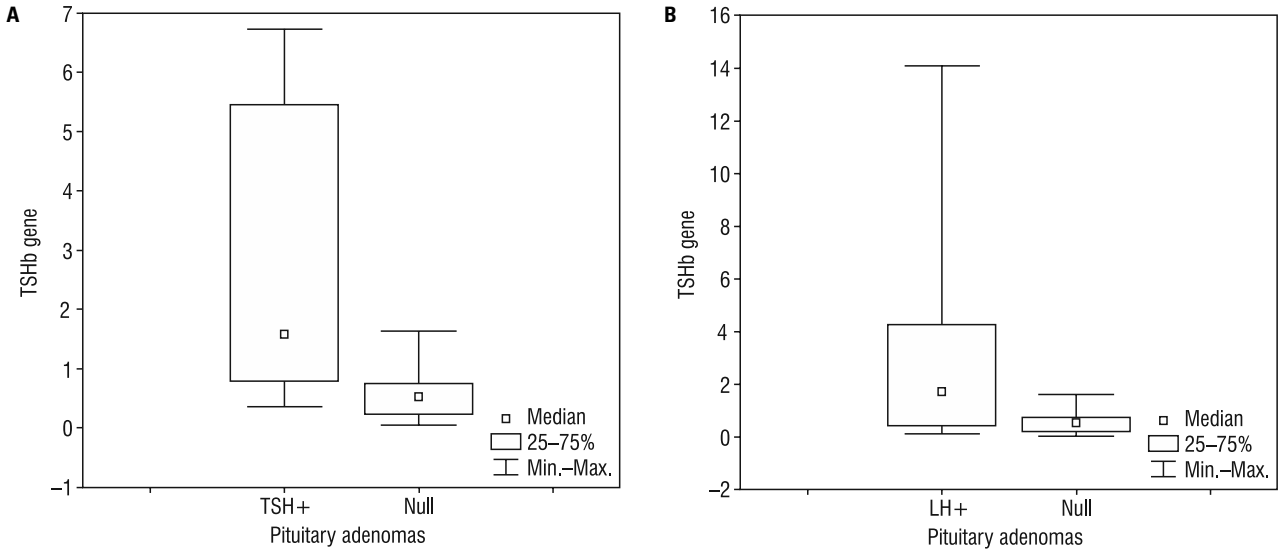


Figure 3. *Comparison of TSHb expression in:* **A.** *TSH+ to IHC negative (null) pituitary adenomas, (U Mann Whitney test, p = 0.04);* **B.** *LH+ to IHC negative (null) pituitary adenomas, (U Mann Whitney test, p = 0.038)*

Rycina 3. *Porównanie ekspresji genu TSHb w:* **A.** *Gruczolakach TSH+ w porównaniu z gruczolakami IHC ujemnymi (null), (test U Manna Whitneya, p = 0.04);* **B.** *Gruczolakach LH+ w porównaniu z gruczolakami IHC ujemnymi (null), (test U Manna Whitneya, p = 0.038)*

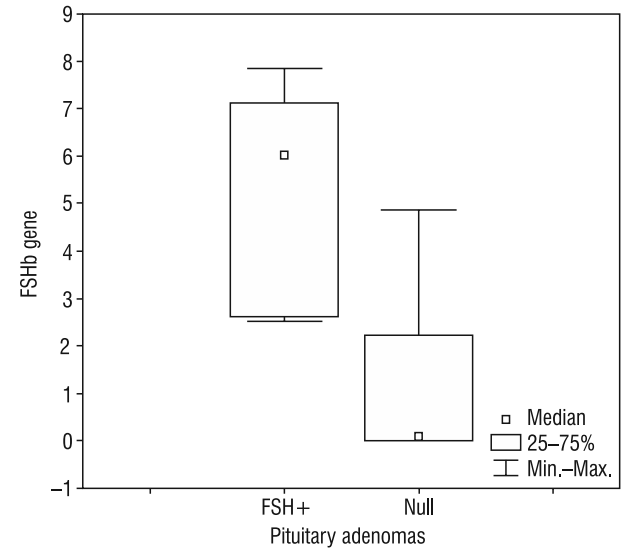


Figure 4. *Comparison of FSHb expression in FSH+ to IHC negative (null) pituitary adenomas, (U Mann Whitney test, p = 0.006)*

Rycina 4. *Porównanie ekspresji genu FSHb w gruczolakach FSH+ w porównaniu z gruczolakami IHC ujemnymi (null), (test U Manna Whitneya, p = 0.006)*

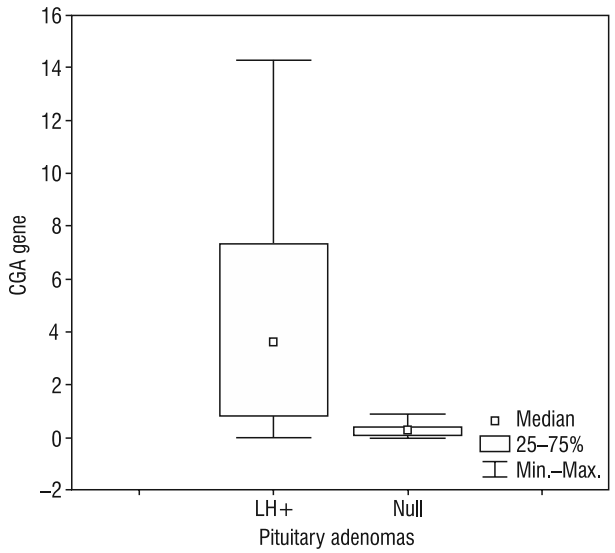


Figure 5. *Comparison of CGA expression in LH+ to IHC negative (null) pituitary adenomas, (U Mann Whitney test, p = 0.0008)*

Rycina 5. *Porównanie ekspresji genu CGA w gruczolakach LH+ w porównaniu z gruczolakami IHC ujemnymi (null), (test U Mann Whitneya, p = 0.0008)*

and 2×, respectively), GH+ (23× and 3.6×, respectively), and in non-functional adenomas (2.4× [8]). Although the differences in *LHb* and *FSHb* gene expression were noticeable between particular types of pituitary adenomas our analysis did not show statistically significant differences between LH+ and FSH+ adenomas.

The last *CGA* gene codes alpha subunit of glycoprotein hormones which is common for FSH, LH, and TSH. In our study we did not observe significant differences in *CGA* expression in particular types of pituitary adenomas that produce glycoprotein hormones.

Conclusions

Our study showed that *GH* is a characteristic gene for somatotropinomas. We drew a similar conclusion for *POMC* gene and adrenocorticotropinomas.

However, the results which we obtained for *PRL*, *TSHb*, *LHb*, *FSHb* and *CGA* genes indicate that evaluation of gene expression is not sufficient for classification of particular subtypes of pituitary adenomas.

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