Pituitary (2019) 22:163–169
https://doi.org/10.1007/s11102-019-00947-x

Filamin A and DRD2 expression in corticotrophinomas

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Published online: 25 February 2019

Abstract

Purpose Filamin A (FLNA) expression is related to dopamine receptor type 2 (DRD2) expression in prolactinomas. Nevertheless, in corticotrophinomas, there are few studies about DRD2 expression and no data on FLNA. Therefore, we evaluated FLNA and DRD2 expression in corticotrophinomas and their association with tumor characteristics.

Methods DRD2 and FLNA expression by immunohistochemistry, using H-score, based on the percentage of positive cells in a continuous scale of 0–300, were evaluated in 23 corticotrophinomas samples from patients submitted to neurosurgery. In six patients, treatment with cabergoline was indicated after non curative surgery.

Results Twenty-two patients were female and one male. Regarding tumor size, 10 were micro and 12 were macroadenomas. DRD2 expression was found in 89% of cases and did not correlate with FLNA expression. Moreover, the response to cabergoline, observed in 33% of the cases, did not correlate with DRD2 nor FLNA expression. FLNA expression was not associated with clinical and tumor characteristics, except for sphenoid sinus invasion.

Conclusions In our cohort of corticotrophinomas, DRD2 expression was not associated with FLNA expression nor to the response to CAB. Nonetheless, FLNA expression could be related to tumor invasiveness.

Keywords Filamin A · DRD2 · Corticotrophinomas · Cushing's disease · Cabergoline

Introduction

Corticotrophinomas, or adrenocorticotropin (ACTH)-secreting pituitary adenomas, account for 13% of pituitary tumors [1], being the most common etiology of endogenous hypercortisolism (Cushing's disease-CD). Although rare, with an estimated incidence of 0.2–1.7/million/year [2], corticotrophinomas are associate with high morbidity and mortality due to systemic complications, especially metabolic syndrome [3]. The disease is more prevalent among women, mainly those between 25 and 40 years. Patients with CD frequently harbor microadenomas, while macroadenomas account for 5–20% of cases [4, 5]. Pituitary surgery, usually via transsphenoidal approach, is the treatment of choice, leading to remission in 73–76% of microadenomas and in 43% of macroadenomas, with recurrence rates between 23 and 33% [6].

In cases without remission, second line therapies include a second surgery, medical treatment, radiotherapy and bilateral adrenalectomy [7]. Medical treatment encompasses pituitary-directed drugs such as pasireotide and cabergoline

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(CAB), adrenal steroidogenesis inhibitors as ketoconazol, and mifepristone, a glucocorticoid receptor blocker [8].

Six studies addressing the issue of CAB treatment after neurosurgical treatment pointed to hormonal control in 25–50% of the cases [9–14]. Among these studies, dopamine receptor subtype 2 (DRD2) expression was only evaluated by Pivonello et al., who found 75% positivity by immunohistochemistry (IHQ) in 15 corticotrophinomas. In vivo treatment with CAB was evaluated in ten cases, and normal urinary cortisol was obtained in four (40%), with a trend towards a correlation between DRD2 expression and CAB response ($p = 0.067$) [9, 10].

Recently, filamin A (FLNA), an actin binding protein involved in receptor anchoring, was implicated in DRD2 expression and dopamine agonist (DA) response in patients harboring prolactinomas [15]. Concerning somatotrophinomas, no correlation was found between FLNA and SSTR2 expression [16]. To the best of our knowledge, no studies addressing FLNA expression in corticotrophinomas had been performed, to date.

Therefore, this study aimed to evaluate DRD2 and FLNA expression levels in corticotrophinomas and their correlation to tumor characteristics and response to CAB.

Materials and methods

Patients

All patients included in the study signed an informed consent, approved by the local ethic committee, being the study performed in accordance with the ethical standards.

This was a retrospective study performed from 2012 to 2017, with a systematic review of medical records, including clinical, laboratory, imaging, pituitary tumor treatments (surgery, drugs, and radiotherapy) and pathological evaluation. We included 23 patients harboring corticotrophinomas followed at our outpatient clinic who underwent pituitary surgery and tumor samples availability for further pathological studies. Tumors were classified as microadenomas (maximum diameter of tumor at MRI < 10 mm) or macroadenomas (≥ 10 mm). A senior radiologist (E.F.C) assessed sellar MRI at diagnosis. Tumor diameters measurements were manually estimated using contrast-enhanced T1 images. Parasellar invasiveness, using Knosp classification (grades 0–2 non-invasive, grades 3 and 4 invasive) [17] and infra-sellar invasiveness, grades 3 and 4, according to Hardy classification [18] were visually addressed (including CT scans, when available). Six patients (four without remission and two with recurrence) received postsurgical treatment with CAB, for at least 4 months, and were classified as responders or non-responders, based on their 24 h urinary cortisol normalization (reference value: 50–310 µg/24 h; AutoDELFIA system, Wallac, Perkin Elmer®, Turku, Finland). The exclusion criterion was radiotherapy at any time before CAB treatment.

Immunohistochemistry

Paraffin-embedded sections were deparaffinized and rehydrated (xylene, alcohol series). Antigen retrieval was performed using 10 mM pH 6.0 citrate buffer solution for 40 min at 95 °C, followed by 20 min cooling at room temperature. Incubation was performed for 30 min at 37 °C with the following: primary antibody, pH 6.0; DRD2 (B-10), 1:400, pH 6.0; and Filamin A—1:800, pH 9.0, previously optimized for our laboratory conditions. Then, an overnight incubation at 4 °C was performed. Signal amplification was performed using Novolink Polymer Detection System (Vision Biosystems™, UK). Normal hypophysis (to Filamin A) and adrenal cortex (to DRD2) were used as controls. A negative control was performed in one tissue sample by substituting the primary antibody with a non-relevant immunoglobulin, with all other steps of reaction performed identically.

We used Filamin A (mouse anti-human filamin monoclonal antibody, clone PM6/317, Millipore® cat: CBL228, lot 2387530, 100 µg conc: 0.1 mg/mL) and dopamine receptor (DRD2 (B-10) mouse monoclonal IgG2a, Santa Cruz® sc-5303 lot J1111, 200 µg/ml, 4 °C).

Two pathologists, JD and FPF, scored DRD2 and FLNA expression using blinded and independent evaluation. Only cytoplasmatic immunostaining was evaluated for DRD2 and FLNA expression. IHC staining was recorded according to four categories: 0 for 'no staining', 1 + for 'light staining', 2 + for 'intermediate staining' and 3 + for 'dark staining'. The percentage of cells at different staining intensities was determined by visual assessment. H-score was calculated bases on the percentage of positive cells in a continuous scale of 0–300 using the formula 1 × (% of 1 + cells) + 2 × (% of 2 + cells) + 3 × (% of 3 + cells) [19]. A strong inter-observer correlation was observed ($R = 0.94$; $P < 0.0001$ for FLNA and $R = 0.8$; $P < 0.001$ for DRD2), and the average H-score was used to represent the protein expression.

Statistical analysis

A frequency distribution was employed to describe categorical variables (number of cases and percentage) and measures of central tendency (mean and median) and variability (lower and upper-quartile and standard deviation) for numerical variables. The Shapiro–Wilk test was used to assess data normality.

To verify the association between the numerical variables and the group variable with two categories, the non-parametric Mann–Whitney U test was applied. Spearman's correlation coefficient was used to indicate direct correlations. The statistical computer program STAT/SE version 14.2 was applied to

perform statistical analyses. The significance level of 5% was adopted for all statistical tests.

Results

Patients

We included 23 patients, 22 females. At diagnosis, the median age was 31 (14–70) years. Eleven patients had microadenomas (one of them only disclosed during surgery), with no identified invasion, and 12 (52%) had macroadenomas. Mean maximal tumor diameter was 1.43 (±0.8) cm, ranging from 0.3 to 5.4 cm. Seven cases presented parasellar invasion (Knosp 3 or 4), and three out of those six also presented sphenoidal sinus invasion Hardy's classification grade 3 (n = 1) and grade 4 (n = 2). All patients underwent pituitary surgery by the transsphenoidal route: 15 cases out of 23 presented remission (65%), four of them with further recurrence. After surgery, six patients, four without remission and two with recurrence, were treated with CAB, being dose titled until 3.5 mg/week, for a median period of 8.5 (4–17) months. Two (33%) patients were considered responders, with 24 h urinary cortisol normalization, and both presented hormonal control escape after 1 and 8 years of treatment. Clinical, laboratory and imaging data are depicted in Table 1.

Immunohistochemistry and tumor characteristics

Routine pituitary hormone immunohistochemistry was performed in all tumor samples and ACTH positivity was found in all cases.

FLNA was evaluated in 23 cases with the median H-score of 5 (interquartile range = 45) and DRD2 was evaluated in 19 cases with the median H-score of 70 (interquartile range = 150). There was no correlation between them (R = 0.11; O = 0.637).

Regarding DRD2 expression, there was no association to tumor size, tumor invasiveness or CAB response. FLNA expression was not correlated to maximal tumor diameter (R = 0.06; P = 0.786) nor microadenomas or macroadenomas (P = 0.89). Although there was no difference in tumors FLNA expression regarding parasellar invasion, its expression was significantly high in tumors with sphenoidal sinus invasion (P = 0.015). FLNA and DRD2 H-score are detailed in Tables 2 and 3. Figure 1 exemplified the high FLNA and DRD2 expression from tumor sample in one case (# 23).

Discussion

In our series, a higher prevalence of female gender (93%), in comparison with literature data [5, 7], was found. Moreover, similarly to our previously published data [5], macroadenomas corresponded to 41% of cases, higher than in literature data [4, 7].

In our study, two out of six patients (33%) responded to CAB, presenting normal 24 h urinary cortisol levels. These data were similar to those reported in the literature, with 25–40% responders in series with more than three patients [9–14]. While in prolactinomas, DRD2 expression is a determinant of dopamine agonist efficacy [20], data are scarce in corticotrophinomas. In our study, moderate to high DRD2 expression was found by immunohistochemistry, in line with the results from Pivonello et al. [9]. In addition, these authors found a tendency toward high expression of DRD2, evaluated by RT-PCR, in CAB responders. In the present cohort, there was no correlation between DRD2 levels and CAB response, although the small size of our sample could influence the statistical results. Additionally, the two CAB responders patients presented hormonal control escape, a phenomenon that has already been described [10, 11].

In our study in corticotrophic tumors, no association between immunostaining of DRD2 and FLNA was found, as well as their expression to CAB response. Regarding FLNA expression and tumor invasiveness, the only association was found with sphenoidal sinus invasion. Therefore, FLNA role in tumor development and aggressiveness should be evaluated in larger clinical series and studies with ATt20 cells. In previous studies with prolactinomas, Peverelli et al. pointed to FLNA role in DRD2 expression and DA response, evaluating DRD2 cytoplasmatic immunostaining in tumor samples and DRD2 membrane expression in cell lines. Nevertheless, no data in prolactinomas regarding FLNA expression and invasiveness are available in literature [15].

Among FLNA functions, in addition to protein anchoring, cell motility is crucial for tumor invasiveness and metastasis [21]. Overexpression of FLNA has been associated with highly metastatic cancers including prostate cancer, melanoma, and neuroblastoma [22–24]. Nevertheless, low FLNA expression was found in other neoplasias, such as melanoma [25] and gastric cancer [26]. Some authors hypothesized that FLNA's dual role depends on where it is located being the cytoplasm location related to tumor-promoting effect, while FLNA located in the nucleus acts as tumor growth suppressor [27]. In our tumor samples, FLNA nuclear location could not be ruled out as we only used cytoplasmatic antibody against FLNA.

In conclusion, in our series cabergoline response was neither associated with DRD2 expression nor with FLNA expression. Nevertheless, we observed intense FLNA expression in corticotrophinomas with infraselar invasiveness, pointing to a possible role of FLNA in corticotrophinoma progression, as has already been described in other neoplasias.

Table 1 Patients' characteristics at diagnosis, cabergoline treatment, FLNA and DRD2 expression (H-score)

ID	Sex	Age (years)	UC (μg/24 h)/ULNR	ACTH[a] (15–46 pg/mL)	MTD (cm)	Infraselar invasiveness	Paraselar invasiveness	CAB mg/w[b]; months	CAB response	HC-score DRD2	H-score FLNA
1	F	27	245[c]/2.72	59	0.7	−	−	−		NA	0
2	F	43	445/1.43	150	2.5	−	+	−		45	0
3	F	70	269/0.86	46	1.5	−	−	−		190	5
4	F	14	1100/3.54	41	NV	−	−	3.5; 6	−	NA	0
5	F	53	430/1.38	37	0.7	−		−		210	60
6	F	30	578/1.86	46	4.0	+	+	−		80	120
7	F	25	624/2.01	34	1.6	−	+	3.5; 9	−	40	0
8	F	25	568/1.83	31	0.7	−	−	−		NA	0
9	F	39	610/1.96	38	0.6	−	−	3.5; 12	−	0	0
10	F	34	156/0.50	83	1.6	−	+	3.5; 17	+	65	0
11	F	24	517/1.66	146	1.2	−	−	−		40	15
12	F	26	1322/4.26	79	0.3	−	−	−		NA	45
13	F	50	395/1.27	111	1.8	−	+	−		115	10
14	F	49	600/1.93	26	0.3	−	−	−		15	80
15	F	47	925/2.98	79	1.1	−	−	−		200	0
16	F	29	724/2.33	51	0.9	−	−	3.5; 8	+	40	50
17	F	14	1207/3.89	68	1.9	−	−	−		70	0
18	F	51	380/1.22	52	0.8	−	−	−		205	10
19	F	31	304/0.98	26	0.7	−	−	−		90	0
20	F	38	671/2.16	55	0.3	−	−	−		20	10
21	M	32	771/2.48	34	0.9	−	−	−		80	0
22	F	20	1186/3.82	234	2.0	+	+	3.5; 4	−	40	30
23	F	17	88/0.28	48[#]	5.4	+	+	−		300	300

NV not visualized, *UC* urinary cortisol, *ULNR* upper limit normal range

[a]Reference: 7.2–63.3 pg/mL

[b]Maximum dose of cabergoline

[c]Reference: <90 μg/24 h

Table 2 Correlation between DRD2 expression (H-score) and tumor size, invasiveness and response to cabergoline

Tumor size	MIC (n = 8)	MAC (n = 11)	*P**
DRD2 median (lower–upper quartile)	60 (17.5–147.5)	70 (40–190)	0.455
DRD2 mean ± SD	82.5 ± 83.1	107.7 ± 86.0	
Infrasellar invasion	No (n = 16)	Yes (n = 3)	*P*
DRD2 median (lower–upper quartile)	67.5 (40–152.5)	80 (40–300)	0.500
DRD2 mean ± SD	89.1 ± 72.8	140 ± 140	
Parasellar invasion	No (n = 12)	Yes (n = 7)	*P*
DRD2 median (lower–upper quartile)	75 (30–195)	65 (40–115)	0.832
DRD2 mean ± SD	96.6 ± 81.6	97.8.0 ± 93.1	
Cabergoline response	No (n = 3)	Yes (n = 2)	*P*
DRD2 median (lower–upper quartile)	40 (0–40)	52.5 (40–65)	0.196
DRD2 mean ± SD	26.7 ± 23.1	52.5 ± 17.7	

MIC microadenoma, *MAC* macroadenoma

*P value calculated from Wilcoxon–Mann–Whitney test

Table 3 Correlation between FLNA expression (H-score) and tumor size, invasiveness and response to cabergoline

Tumor size	MIC (n = 12)	MAC (n = 11)	*P**
FLNA median (lower–upper quartile)	5 (5–80)	5 (0–300)	0.896
FLNA mean ± SD	21.2 ± 29.1	41.8 ± 90.6	
Infrasellar invasion	No (n = 20)	Yes (n = 3)	*P*
FLNA median (lower–upper quartile)	0 (0–12.5)	100 (30–300)	0.015
FLNA mean ± SD	14.2 ± 24.1	143.3 ± 140.1	
Parasellar invasion	No (n = 16)	Yes (n = 7)	*P*
FLNA median (lower–upper quartile)	2.5 (0–30)	10 (0–100)	0.478
FLNA mean ± SD	17.2 ± 26.1	62.8 ± 110.5	
Cabergoline response	No (n = 4)	Yes (n = 2)	*P*
FLNA median (lower–upper quartile)	0 (0–15)	25 (0–50)	0.411
FLNA mean ± SD	7.5 ± 15	25 ± 35.4	

MIC microadenoma, *MAC* macroadenoma

*P value calculated from Wilcoxon–Mann–Whitney test

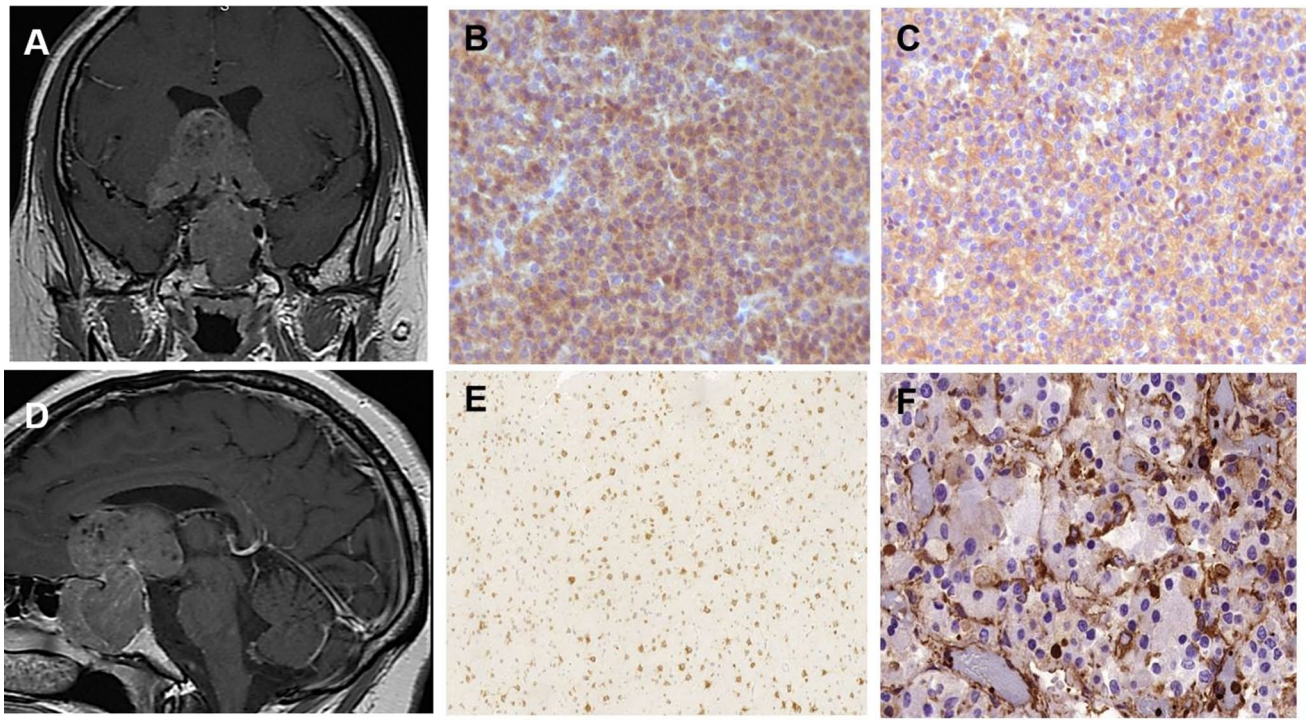

Fig. 1 Sellar MRI depicting a 6.5 cm pituitary tumor, after two surgeries, impinging optical chiasma, with parasellar and infrasellar invasion in T1-weighted coronal (**a**) and sagittal view (**d**), both with gadolinium enhancement. DRD2 expression H-score was 300 (**b**), comparing to positive control (**e**) and FLNA expression H-score was also 300 (**c**), comparing to positive control (**f**), ×400

Acknowledgements We specially thank Alda Wakamatsu for her carefully help in immunohistochemistry reactions. This work was supported by the National Council of Scientific and Technological Development (CNPq), number 162014/2013-9 and Federico Foundation.

Compliance with ethical standards

Conflict of interest The authors declare that have no conflict of interest.

Ethical approval This study was approved by the ethics committee and was performed in accordance with the ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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