Expression profiles of the glucose-dependent insulinotropic peptide receptor and LHCGR in sporadic adrenocortical tumors

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Abstract

Glucose-dependent insulinotropic peptide receptor (GIPR) and LHCGR are G-protein-coupled receptors with a wide tissue expression pattern. Aberrant expression of these receptors has rarely been demonstrated in adult sporadic adrenocortical tumors with a lack of data on pediatric tumors. We quantified the *GIPR* and *LHCGR* expression in a large cohort of 55 patients (25 children and 30 adults) with functioning and non-functioning sporadic adrenocortical tumors. Thirty-eight tumors were classified as adenomas whereas 17 were carcinomas. *GIPR* and *LHCGR* expression were analyzed by real-time PCR and normal human pancreatic and testicular tissue samples were used as positive controls. Mean expression values were determined by fold increase in comparison with a normal adrenal pool. *GIPR* mRNA levels were significantly higher in

adrenocortical carcinomas than in adenomas from both pediatric and adult groups. *LHCGR* expression was similar in both carcinomas and adenomas from the pediatric group but significantly lower in carcinomas than in adenomas from the adult group (median 0.06 and 2.3 respectively, $P < 0.001$). *GIPR* was detected by immunohistochemistry in both pediatric and adult tumors. Staining and real-time PCR results correlated positively only when *GIPR* mRNA levels were increased at least two-fold in comparison with normal adrenal expression levels. In conclusion, *GIPR* overexpression was observed in pediatric and adult adrenocortical tumors and very low levels of *LHCGR* expression were found in all adult adrenocortical carcinomas.

Journal of Endocrinology (2009) **200**, 167–175

Introduction

The molecular mechanisms underlying the pathogenesis of primary adrenocortical disorders have recently begun to be unraveled (Koch *et al.* 2002), with several genetic alterations being implicated in the development of different forms of adrenocortical hyperplasia and tumors (Luton *et al.* 1998, Kirschner *et al.* 2000, Wilkin *et al.* 2000, Latronico *et al.* 2001, Fragoso *et al.* 2003, Bourdeau *et al.* 2004, Pinto *et al.* 2005, West *et al.* 2007, Else *et al.* 2008). In both hyperplastic and adrenocortical tumor tissues, aberrant expression of several G-protein-coupled receptors (GPCRs) has been identified, placing adrenal cells under the stimulation of trophic factors not negatively regulated by glucocorticoids (Lacroix *et al.* 2001, 2004).

GPCRs constitute a large and diverse family of proteins, whose primary function is to transduce extracellular stimuli into intracellular signals (Kroeze *et al.* 2003). The glucose-dependent

insulinotropic peptide receptor (GIPR) is a member of the GPCR and of the secretin–vasoactive intestinal peptide receptor sub-family (Usdin *et al.* 1993). *GIPR* is expressed in human pancreatic β -cells but not in normal adrenocortical tissue (Lacroix *et al.* 1992, 2004, Chabre *et al.* 1998, Lebrethon *et al.* 1998). During the last decade, aberrant *GIPR* expression was demonstrated in ACTH-independent macronodular adrenal hyperplasia and adrenocortical adenomas (Reznik *et al.* 1992, Chabre *et al.* 1998, Lebrethon *et al.* 1998, Luton *et al.* 1998, N'Diaye *et al.* 1998, 1999, Tsarakis *et al.* 2001, Noordam *et al.* 2002). More recently, overexpression has also been shown in the adrenals of patients with Cushing's disease and in primary pigmented nodular adrenocortical disease (Swords *et al.* 2005). However, these findings have not been confirmed by other studies (N'Diaye *et al.* 1998, Antonini *et al.* 2006).

The LHCGR is a GPCR mainly involved in the regulation of ovarian and testicular functions (Ascoli *et al.* 2002), which binds

to human chorionic gonadotropin (hCG) and LH (McFarland *et al.* 1989). hCG has a wide distribution throughout fetal tissues and targets non-gonadal tissues in adults. In addition, a broad LHCGR expression pattern has been demonstrated in several human fetal tissues, including adrenal cortex (Abdallah *et al.* 2004). A preliminary study in adult and pediatric patients with non metastasizing or metastasizing/recurrent adrenocortical tumors conducted in our institution revealed low levels of tumoral LHCGR expression in the majority of the adult patients with metastasizing disease. By contrast, most pediatric patients with adrenocortical tumors presented with high levels of tumoral LHCGR expression (Barbosa *et al.* 2004). The physiological role of the LHCGR expression in normal human adrenal glands and in adrenocortical tumors is not clear yet.

Differently from adults, pediatric adrenocortical tumors with apparent poor prognosis on the basis of histopathological features may often have a better clinical outcome. Taken together, these molecular and clinical data suggest that adrenocortical tumorigenesis is distinct in children and adults (Mendonca *et al.* 1995, Wieneke *et al.* 2003, Almeida *et al.* 2008).

Adrenocortical tumors in children and adolescents are rare but tend to cluster around certain areas (Almeida & Latronico 2007, West *et al.* 2007). The incidence of adrenocortical tumors in children from the southern region of Brazil is noticeably high, approximately 10–15 times greater than the worldwide incidence (Sandrini *et al.* 1997). The germ line mutation R337H in the tetramerization domain of the *P53* tumor suppressor has been found to be extremely frequent in this population, being identified in 78–97% of pediatric adrenocortical tumors in Southern Brazil (Latronico *et al.* 2001, Ribeiro *et al.* 2001). This mutation was not restricted to the pediatric group, as 13.5% of adults with adrenocortical tumors also had this *P53* defect (Latronico *et al.* 2001), and a founder effect for this mutation was later identified in Brazilian patients with adrenocortical tumors (Pinto *et al.* 2004).

In the present study, we quantified *GIPR* and *LHCGR* expression levels in sporadic adrenocortical tumors from a large Brazilian cohort of pediatric and adult patients with and without the R337H mutation of *P53*.

Patients and methods

This study was approved by the Ethics Committee of Hospital das Clinicas, Sao Paulo, Brazil, and written informed consent was obtained from normal individuals, all patients or their parents. We studied 55 unrelated Brazilian patients with adrenocortical tumors; 20 children (aged 0.9–9.0 years), 5 adolescents (aged 15–18 years), and 30 adults (aged 22–66 years).

The pre-surgical hormonal evaluation of all patients included peripheral blood determination of LH, FSH, testosterone, estradiol, ACTH, dehydroepiandrosterone sulfate, dehydroepiandrosterone, androstenedione, 11-deoxycortisol, aldosterone, plasmatic renin activity, and 24 h urinary cortisol. Serum cortisol levels were determined in basal conditions and after overnight

administration of 1.0 mg dexamethasone in adults or 10 µg/kg in children below 40 kg weight. All patients also had pre-surgical electrolytes determination. Out of the 55 patients studied, 49 had functioning tumors (19 androgen producing, 16 glucocorticoid producing, 12 androgen/glucocorticoid producing, 1 estrogen/glucocorticoid producing, and 1 inhibin-A producing), whereas 6 adult patients had non-functioning tumors.

The pathological diagnosis of malignancy was established according to MacFarlane staging $\geq$ III (Macfarlane 1958, Sullivan *et al.* 1978) associated with the clinical follow up in both groups in order to characterize long distance metastasis, survival range, and death. The follow-up ranged from 1.4 to 13 years (mean, 5.3 years; Tables 1 and 2).

In our cohort of patients, 20 children, 5 adolescents (pediatric group), and 23 adults (adult group) had been previously screened for the R337H mutation of *P53* tumor suppressor gene. This mutation had been identified in 17 patients (68%) in the pediatric group (15 children and 2 adolescents) and 2 patients (8.7%) in the adult group (Latronico *et al.* 2001, Pinto *et al.* 2005).

LHCGR expression had been previously studied in 18 (15 adults and 3 children) patients of our cohort using dot-blot hybridization and observed to be low in some of the patients with metastasizing carcinoma (Barbosa *et al.* 2004).

Quantitative expression of *GIPR* and *LHCGR*

All patients underwent unilateral adrenalectomy, except patient 21 (Table 1) who was submitted to bilateral adrenalectomy due to tumor recurrence in the remaining adrenal 2 years after the first surgery. Tumor samples were obtained from the core of the excised tumors to minimize possible contamination by normal surrounding tissue. Necrotic and hemorrhagic areas were also avoided. Tissue fragments were immediately stored in liquid nitrogen until RNA extraction.

RNA extraction, cDNA synthesis, and reverse transcription-PCR

Total RNA was isolated from frozen tissue using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription (RT) was performed in 5 µg total RNA of each sample using Multiscribe from a High-capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) in a 50 µl total reaction.

Quantitative real-time PCR was carried out in the Applied Biosystems 7000 real-time PCR System. A TaqMan Gene Expression Assay (Applied Biosystems) was especially designed to amplify LHCGR. *GIPR* amplification was performed using available commercial primers and a probe (Assay ID Hs006092_m1, Applied Biosystems). LHCGR amplification was performed with the following pair of primers 5'-GCACAATGGAGCCTTCCGT-3', 5'-GGCCTGC-AATTTGGTGGAA-3', and the probe 5'-CCGAAAACCT-TGGATATTT-3'. β -actin (assay ID-4326315E, Applied Biosystems) was chosen as the internal control. Multiplex reactions consisted of 12.5 µl 2× TaqMan Universal PCR

Table 1 Clinical, histological and molecular data from 25 pediatric patients with adrenocortical tumors

	Age (year)	Sex	Endocrine syndrome	Tumor weight (g)	Weiss score	MacFarlane staging	Follow up (year)	Diagnosis histological	P53 R337H	LHCGR expression	GIPR expression	GIPR immunohisto chemistry
Patient (n)												
1	1·3	M	V	30	IV	I	11·0	Adenoma	+	1·8	4·1	—
2	2·3	F	V	5	V	I	2·4	Adenoma	+	2·8	4·6	+
3	0·9	F	V/C	60	III	II	1·4	Adenoma	+	6·7	12·1	+
4	2·2	M	V	90	IV	II	10·3	Adenoma	+	4·6	5·4	—
5	2·1	F	V	5	II	I	8·0	Adenoma	+	1·4	1·1	+
6	2·1	M	V	135	V	II	6·9	Adenoma	+	2·4	53·7	+
7	1·3	M	V/C	40	I	I	7·2	Adenoma	+	0·3	6·8	+
8	2·5	F	V	55	VII	II	4·5	Adenoma	+	7·9	6·1	+
9	1·7	F	V	NA	III	II	10·0	Adenoma	—	4·2	11·8	NA
10	9	M	C	20	II	I	4·9	Adenoma	—	0·5	0·8	+
11	6	F	V/C	45	II	I	7·0	Adenoma	—	8·7	6·4	NA
12	2·8	F	V	30	II	I	11·0	Adenoma	+	47·7	1·3	—
13	2·2	F	V/C	55	II	II	3·0	Adenoma	—	0·5	0·8	+
14	2·5	F	V	10	I	I	2·0	Adenoma	+	19·1	2·1	+
15	2·1	F	V	20	I	I	1·5	Adenoma	+	43·5	0·8	—
16	1·6	F	V	NA	NA	I	5·0	Adenoma	+	33·5	8·2	—
17	18	F	C	20	III	I	6·5	Adenoma	—	1·1	0·3	+
18	2·6	M	V	70	VII	IV	1·4*	Carcinoma	+	4·3	0·9	+
19	0·9	F	V	135	VII	IV	2·0	Carcinoma	+	13·1	6·8	+
20	2	F	V/C	55	VII	IV	2·4*	Carcinoma	—	2·3	58·1	+
21	2·6	M	V	NA	VII	IV	9·6	Carcinoma	+	15·6	130·8	+
22	15	F	V/C	1230	VII	IV	2·9*	Carcinoma	+	8·5	11·5	+
23	17	M	f/C	165	VII	III	1·7	Carcinoma	—	0·007	1·3	+
24	18	F	V/C	1000	VII	IV	1·8*	Carcinoma	+	28·6	131·3	+
25	17	F	V	825	IV	III	3·2	Carcinoma	—	0·06	24·5	+
Normal adrenal										2·5	0·7	—
Normal testis										45·3		
Normal pancreas											74·4	+

M, male; F, female; C, Cushing syndrome; V, virilization; f, feminization; NA, not available; *, deceased. (—) Absent GIPR stain; (+) to GIPR stains more than 50% of adrenal tumor cells or pancreatic normal cells.

Table 2 Clinical, histological, and molecular data from 30 adult patients with adrenocortical tumors

	Age (year)	Sex	Endocrine syndrome	Tumor weight (g)	Weiss score	MacFarlane staging	Follow up (year)	Diagnosis histological	P53 R337H	LHCGR expression	GIPR expression	GIPR immunohistochemistry
Patient (n)												
26	37	F	C	20	II	I	1·5	Adenoma	—	2·4	1·4	—
27	27	F	C	5	II	II	8·1	Adenoma	+	2·3	0·5	—
28	46	F	NF	30	I	I	2·3	Adenoma	NA	0·1	1·6	+
29	35	F	V/C	40	III	I	4·0	Adenoma	—	30	3·3	+
30	29	F	C	20	I	I	9·8	Adenoma	—	2·1	4·0	—
31	38	F	C	45	I	I	11·6	Adenoma	—	7·8	2·4	+
32	26	F	C	30	I	I	4·3	Adenoma	—	4·5	1·7	—
33	50	F	V	35	III	I	5·5	Adenoma	—	0·3	1·4	—
34	37	F	C	15	I	I	4·7	Adenoma	—	3·5	0·1	NA
35	47	F	C	5	I	I	6·3	Adenoma	—	0·6	0·4	—
36	49	M	NF	10	I	I	2·5	Adenoma	NA	1·3	1·1	+
37	40	F	NF	10	I	I	10·5	Adenoma	—	0·7	1·3	—
38	64	F	C	15	I	I	2·0	Adenoma	NA	0·3	0·2	+
39	39	F	C	10	I	I	8·2	Adenoma	NA	7·0	1·2	+
40	27	F	C	20	I	I	NA	Adenoma	—	1·6	0·6	—
41	37	F	C	25	I	I	9·4	Adenoma	—	18	4·7	+
42	45	F	NF	20	NA	I	NA	Adenoma	NA	2·6	1·3	—
43	28	F	V	150	II	II	3·0	Adenoma	NA	0·3	61·5	+
44	24	F	C	15	II	I	8·7	Adenoma	—	0·6	0·3	+
45	41	F	C	NA	I	I	13	Adenoma	—	11·1	0·7	NA
46	66	F	NF	55	IV	II	1·6	Adenoma	—	0·3	21·3	—
47	29	M	C	665	VIII	IV	1·4*	Carcinoma	—	0·3	4·1	+
48	33	M	NF	NA	V	III	2·3	Carcinoma	NA	0·004	5·4	NA
49	30	M	IN	330	IV	IV	2·0*	Carcinoma	—	0·4	19·5	+
50	45	F	V/C	280	VII	III	6·5	Carcinoma	—	0·004	2·1	+
51	49	F	V/C	930	IV	III	7·0	Carcinoma	—	0·06	1·7	+
52	23	F	V/C	555	VI	IV	2·4*	Carcinoma	+	0·06	20·5	+
53	29	F	V/C	550	IV	IV	3·5*	Carcinoma	—	0·13	2·6	+
54	22	F	V	374	VII	IV	1·8*	Carcinoma	—	0·01	7·3	NA
55	44	F	V	810	VIII	III	4·0*	Carcinoma	—	0·01	1·1	+
Normal adrenal										2·5	0·7	—
Normal testis										45·3		
Normal pancreas											74·4	+

M, male; F, female; C, Cushing syndrome; V, virilization; f, feminization; IN, inhibin; NF, non-functioning; NA, not available; *, deceased (—) Absent GIPR (—) Absent GIPR stain; (+) to GIPR stains more than 50% of adrenal tumor cells or pancreatic normal cells.

master mix, 1.25 µl each 20× assay on demand, 1.5 µl cDNA, and water to complete 25 µl final volume. PCR parameters were 50 °C for 2 min, 95 °C for 10 min followed by 50 cycles at 95 °C for 15 s, and 60 °C for 1 min.

Validation experiments were performed to verify that the amplification efficiency of the controls was similar to that of the target genes.

A cycle threshold (C_t) value in the linear range of amplification was selected for each sample in triplicate and normalized to β -actin expression levels. The relative expression levels were analyzed using the $2^{-\Delta\Delta C_t}$ method (Livak & Schmittgen 2001), where the $\Delta\Delta C_t$ is the difference between the selected ΔC_t value of a particular sample and the ΔC_t of a pool in 61 normal adrenals from autopsies (Clontech, Palo Alto, CA, USA). The mean expression of the target genes in the normal adrenals pool was assigned an expression value of 1.0 and fold increases in the expression levels in each tumor sample was determined by comparison.

Normal human tissue samples from adrenal glands, pancreas, and testis were obtained during surgical resections of kidney tumors, pancreatic cysts, and gonads respectively, and were used as controls.

GIPR immunohistochemistry

Considering that GIPR is ectopically expressed in adrenal tissue, we also analyzed its expression by immunohistochemistry.

All paraffin-embedded adrenal tumor tissue samples were processed overnight in Autotechnicon (Technicon, New York, NY, USA). Histological sections of 4 µm were laid on glass slides, which had been previously treated with 3-aminopropyltriethoxysilane, warmed at 60 °C overnight, deparaffinized in xylol and re-hydrated with decreasing concentrations of ethanol. A histological section from each tumor fragment was stained with hematoxylin-eosin. Endogenous peroxidase was blocked with hydrogen peroxide for the immunohistochemistry reactions. In order to retrieve the antigens, the sections were immersed in a citrate buffer (0.01 mol/l, pH 6.0) and warmed in a pressure cooker for 3–5 min. The following primary antibody was used: GIPR OPA1-15060 (Bioreagents, Golden, CO, USA) with epitope in N-terminal extracellular region. Reactivity was detected through incubation with goat anti-rabbit (GIPR) secondary antibody and a system of peroxidase-conjugated polymer (Envision +, Dako, Dakocytomation, Carpinteria, CA, USA), using 3,3'-diaminobenzidine as the chromogen. Counterstaining was carried out with Harris hematoxylin.

Immunohistochemical experiments were performed in duplicate and samples incubated with primary antibody alone served as negative control for the reaction. Normal adrenal tissue, without expressive GIPR staining, was used as a negative control sample for these experiments. Sections of pancreatic tissue samples were used as positive GIPR expression control. The

Figure 1 Immunohistochemical analysis of human adrenocortical tumors using a polyclonal antibody to GIPR. (A) and (B) show samples with negative GIPR staining at 100× and 400× magnification respectively. (C) and (D) display a large group of intensively GIPR positive cells at 100× and 400× magnification respectively.

reaction was considered positive when neoplastic cells showed unequivocal staining (>50% of cells in 100× and 400× magnification) in relation to the background staining (Fig. 1).

Statistical analysis

GIPR and *LHCGR* expression levels from all tissue analyzed were compared by the Kruskal–Wallis test. A $P<0.05$ was considered significant. Data are presented as median and range for each group. The Spearman test was used to establish correlation between the receptor expression, patient clinical aspects, hormonal levels, and the presence of *P53* mutation (R337H).

Results

GIPR and LHCGR expression

GIPR and *LHCGR* were expressed in normal and adrenocortical tumor samples (Table 3). We verified *GIPR* overexpression in adrenocortical carcinomas compared with adenomas from both pediatric (median = 18.1, ranging from 0.9 to 131.3) and adult groups (median = 4.1, ranging from 1.1 to 20.5; Fig. 2). The highest *GIPR* mRNA levels were detected in two large functioning adrenocortical carcinomas in the pediatric group (Table 2).

LHCGR mRNA levels were similar in adrenocortical adenomas and carcinomas from the pediatric group (Table 3). By contrast, *LHCGR* mRNA levels were significantly lower in adrenocortical carcinomas (median = 0.06, ranging from 0.004 to 0.4; $P<0.001$) than in adenomas from the adult group (Fig. 3).

Six adults and four children diagnosed with carcinoma died during the follow-up, ranging from 1.4 to 4 years, and 1.4 to 2.9 years respectively. *GIPR* or *LHCGR* expression levels in this group of tumors were not different from the rest of carcinomas.

We did not find a correlation between *GIPR* or *LHCGR* expression and the presence of the R337H mutation in *P53* ($P>0.05$). We also did not find any correlation between

Figure 2 Levels of *GIPR* mRNA expression in 55 benign and malignant adrenocortical tumors compared with those of eight normal human adrenal glands (NL). ABT, adults with benign tumors; AMT, adults with malignant tumors; CBT, children with benign tumors; CMT, children with malignant tumors.

GIPR or *LHCGR* expression levels in the tumor and pre-surgical hormonal levels ($P>0.05$).

Immunohistochemistry for *GIPR* was performed by two expert pathologists in 49 adrenocortical tumors (Tables 1 and 2). *GIPR* overexpression (>two-fold when compared with normal adrenal tissue) detected by real-time PCR was observed in most adrenocortical tumors from pediatric and adult groups. Immunohistochemistry was positive for *GIPR* in both pediatric and adult tumors in which mRNA levels detected by real-time PCR indicated *GIPR* overexpression.

Discussion

GIPR and *LHCGR* belong to the GPCR superfamily (Usdin *et al.* 1993, Ascoli *et al.* 2002). Aberrant expression of GPCRs in the adrenal cortex has been demonstrated in several variants of ACTH-independent Cushing's syndrome associated with adrenocortical hyperplasia and tumors (Chabre *et al.* 1998, Luton *et al.* 1998, N'Diaye *et al.* 1998, 1999, Lacroix *et al.* 1999b, 2001, 2004, Mircescu *et al.* 2000, Bourdeau *et al.* 2007), and clinical evidence suggests their implication in abnormal

Table 3 Median and range of *GIPR* and *LHCGR* mRNA levels in pediatric and adult adrenocortical tumors

	GIPR expression		LHCGR expression	
	Pediatric	Adult	Pediatric	Adult
Adrenal tissue				
Adenoma	4.6 (0.3–53.7)	1.3 (0.1–61.5)	4.3 (0.3–47.7)	2.3 (0.1–30)
Carcinoma	18.1 (0.9–131.3)	4.1 (1.1–20.5)	6.4 (0.007–28.6)	0.06 (0.004–0.4)
	$P<0.05^a$	$P<0.001^b$	$P=ns$	$P<0.001^c$
Normal	0.7 (0.1–1.4)		2.5 (1.3–9.6)	

Statistical analysis of *GIPR* expression (adenoma × carcinoma) in pediatric group (a) and in adult group (b). Statistical analysis of *LHCGR* expression (adenoma × carcinoma) in adult group (c). ns, non-significant.

Figure 3 Levels of *LHCGR* mRNA expression in 55 adrenocortical tumors compared with those of eight normal human adrenal glands (NL). ABT, adults with benign tumors; AMT, adults with malignant tumors; CBT, children with benign tumors; CMT, children with malignant tumors.

regulation of steroidogenesis (Lacroix *et al.* 1992, 1999a, Tsagarakis *et al.* 2001, Feelders *et al.* 2003, Goodarzi *et al.* 2003). However, it remains uncertain whether these receptors are expressed differently in benign or malignant tumors (Mircescu *et al.* 2000, Lacroix *et al.* 2001, 2004). Interestingly, ectopic GIPR expression in the adrenal cortex has been previously demonstrated in food-dependent Cushing syndrome associated with benign adrenal tumors; although, this was not observed in a small cohort of malignant ones (Groussin *et al.* 2002). Recently, Mazzuco *et al.* (2006a,b) used an *in vivo* cell transplantation model in mice to show that aberrant expression of wild-type GIPR and LHCGR were sufficient to initiate the formation of benign adrenocortical tumors and hyperplastic adrenal tissue associated with Cushing's syndrome features. No mutations in the coding or promoter regions of GIPR have been identified so far in patients with adrenocortical tumors and the molecular mechanisms responsible for the ectopic GIPR expression in the adrenal gland remain unclear (Antonini *et al.* 2002, 2004, Lampron *et al.* 2006). In this study, we quantified GIPR and LHCGR expression in a large Brazilian cohort of adults and children with adrenocortical tumors to analyze their expression patterns in this complex entity.

We identified a substantial variability and overlap of GIPR expression levels between benign and malignant tumors of both children and adults, although median GIPR mRNA levels were significantly higher in adrenocortical carcinomas than in adenomas from both pediatric and adult groups. However, the clinical behavior of adrenal tumors which overexpressed GIPR was similar to those which did not, suggesting that routine assessment of GIPR expression levels in adrenal tumors might not be useful to predict tumor outcome.

Overexpression of LHCGR has been demonstrated in humans with benign adrenal tumors; LHCGR expression was elevated in more than half of aldosterone-secreting adenomas, implicating a role for LHCGR in the pathophysiology of this disease (Saner-Amigh *et al.* 2006). In a previous study using RT-PCR and dot-blot hybridization, we found low LHCGR expression levels in adrenocortical carcinomas (Barbosa *et al.* 2004). In the present study, using a more sensitive method, we confirmed low levels of LHCGR expression in adrenocortical carcinomas from adults and adolescents, suggesting a correlation between low LHCGR expression and aggressive tumor behavior in these patients.

The tendency towards lower levels of LHCGR expression in adrenocortical carcinomas from adolescents and adults compared with those from children supports the hypothesis that adrenocortical tumorigenesis follows different pathways in these groups.

In conclusion, GIPR overexpression was observed in pediatric and adult adrenocortical tumors and could not indicate clinical prognosis. On the other hand, very low levels of LHCGR expression were detected in all adult adrenocortical carcinomas, and in two adolescents, suggesting an association between malignancy and LHCGR expression pattern in these age ranges.

Declaration of interest

There is no conflict of interest between the authors and the funding agencies that would prejudice the impartiality of this work.

Funding

This work was supported in part by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP Grants 03/07449-1 to M H S C and 04/15046-7 to B B M) and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Grants 300828/2005-5 to B B M and 300469/2005-5 to A C L).

Acknowledgements

The authors thank the staff at Laboratório de Hormônios e Genética Molecular LIM/42. We also thank Dr Alexander Augusto Jorge and Dr Silvia Correa Souza Leao for the statistical analysis and Dr Bruno Ferraz-de-Souza for English revision of the manuscript.

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Received in final form 23 October 2008

Accepted 25 October 2008

Made available online as an Accepted Preprint

29 October 2008