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(Article begins on next page)

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Coexisting Prolactinoma and Primary Aldosteronism: Is There a Pathophysiological Link?

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Abstract

Context:

Coexisting prolactinoma-primary aldosteronism (PA) is infrequently reported.

Objective:

The objective of the study was to identify patients with prolactinoma-PA and test the hypothesis that elevated prolactin (PRL) concentrations play a role in PA pathogenesis.

Setting and Design:

Hyperprolactinemia/prolactinoma was diagnosed in PA patients from two referral centers (Munich, Germany, and Turin, Italy) and in essential hypertensive (EH) patients from one center (Turin). PRL receptor (*PRLR*) gene expression was determined by microarrays on aldosterone-producing adenomas and normal adrenals and validated by real-time PCR. H295R adrenal cells were incubated with 100 nM PRL, and gene expression levels were determined by real-time PCR and aldosterone production was quantified.

Results:

Seven patients with prolactinoma-PA were identified: four of 584 and three of 442 patients from the Munich and Turin PA cohorts, respectively. A disproportionate number presented with macroprolactinomas (five of seven). There were five cases of hyperprolactinemia with no cases of macroprolactinoma of 14 790 patients in a general EH cohort. In a population of PA patients case-control matched 1:3 with EH patients there were two cases of hyperprolactinemia of 270 PA patients and no cases in the EH cohort (n = 810). *PRLR* gene expression was significantly up-regulated in the aldosterone-producing adenomas compared with normal adrenals (1.7-fold and 1.5-fold by microarray and real-time PCR, respectively). In H295R cells, PRL treatment resulted in 1.3-fold increases in *CYP11B2* expression and aldosterone production.

Conclusion:

Elevated PRL caused by systemic hyperprolactinemia may contribute to the development of PA in those cases in which the two entities coexist.

Primary aldosteronism (PA) is the leading cause of secondary hypertension that affects 4.3% of patients in the general hypertensive population and 9.5% of patients referred to hypertension units (1). It is characterized by the constitutive hypersecretion of aldosterone autonomous of the renin-angiotensin system. The two most frequent subtypes of PA are either an aldosterone-producing adenoma (APA) or bilateral adrenal hyperplasia (BAH) that are diagnosed by a multistep protocol following The Endocrine Society guidelines (2). Subtype differentiation offers a targeted therapy of either the surgical removal of an APA or of pharmacological treatment with a mineralocorticoid antagonist for BAH (3).

Hyperprolactinemia is diagnosed with a single measurement of serum prolactin (PRL) (4). Prolactinomas are the most common type of pituitary tumor, with a reported population prevalence range of 6–50 per 100 000 (5, 6). In a large cohort of 1607 dopamine agonist-treated hyperprolactinemia patients, the prevalence

was 10 per 100 000 in men (with no apparent age group peak frequency) and 30 per 100 000 in women (with a peak frequency in the 25–34 year age group) (7). In women with reproductive disorders, the incidence of hyperprolactinemia has been reported to be as high as 9%–17% (8).

Prolactinomas are classified as microprolactinomas (≤ 1 cm) that are more common in women and macroprolactinomas (>1 cm) that are more common in men (9). Giant prolactinomas (>4 cm) are usually associated with very high prolactin levels (1000–100 000 $\mu\text{g/L}$) and account for 2%–3% of all PRL-secreting tumors and are 9 times more frequent in men (10). Malignant prolactinomas are extremely rare and are thought to arise from the transformation of initially large, benign adenomas (11). In some cases, prolactinomas occur as part of the autosomal-dominant genetic disorder, multiple endocrine neoplasia type 1 (MEN1) in which they develop in association with additional tumors of the parathyroid and pancreatic islet cells (12). The primary treatment of prolactinoma patients is with dopamine agonists to normalize prolactin levels and reduce tumor mass (13).

We were intrigued by a recurring number of cases of coincident prolactinoma-PA from the German Conn Registry. The coexistence of these two syndromes has been infrequently reported in the literature (14–20), and a putative role for hyperprolactinemia in PA remains to be specifically addressed.

Herein we report the largest-ever case series of coincident prolactinoma-PA recruited through two different referral centers. The Munich (Germany)-Turin (Italy) prolactinoma-PA case series presents a disproportionate occurrence of macroprolactinoma coexisting with PA, thereby raising the hypothesis that elevated PRL levels arising from systemic hyperprolactinemia may play a pathophysiological role in PA. We show that the PRL receptor (*PRLR*) gene is up-regulated in APA compared with normal adrenals, and high PRL concentrations, comparable with those found in macroprolactinoma patients, result in the up-regulation of the aldosterone synthase gene (*CYP11B2*) and an increased production of aldosterone in the NCI H295R adrenal cell line.

Materials and Methods

Cases in the literature

A MEDLINE database search was performed using the key words, prolactinoma and (hyper-)aldosteronism; aldosteronism and pituitary and macroadenoma; and aldosteronism and pituitary and microadenoma and aldosterone and adenoma and prolactin. Eligible cases of coincident PA and prolactinoma were identified, relying on the diagnostic criteria of each reporting center for both conditions, which varied markedly. Reported cases were excluded if the authors considered the diagnosis of either PA or prolactinoma uncertain.

Diagnosis of PA and prolactinoma

Protocols were approved by local ethics committees, and all participants gave their written informed consent.

Munich

The Munich center of the German Conn Registry retro- and prospectively documents all PA patients treated in the in- or outpatient clinic after 1990. Data collection and yearly follow-ups of retro- and prospectively recruited patients started in 2006. The diagnostic criteria for PA, used by our center, have been described elsewhere (21, 22). The total number of patients with PA treated since 1990 is 584 (294 from 1990 to 1997 and 290 since 2008).

Hyperprolactinemia was clinically suspected and diagnosed by serum PRL levels greater than 20 ng/mL or greater than 500 $\mu\text{U/mL}$ using an immunoassay (ADVIA Centaur Prolactin; Siemens Healthcare). After the

diagnosis, patients underwent magnetic resonance imaging scanning of the pituitary. The patients of the German Conn Registry are not routinely screened for elevated PRL levels or anterior hypopituitarism.

Turin

Diagnosis of PA was established and subtype differentiation performed as described in detail elsewhere (23). The total number of PA subjects treated in Turin since 1991 is 442. The patients of the Torino Hypertension Unit are not routinely screened for elevated PRL levels. Reasons for screening are oligomenorrhea/amenorrhea or galactorrhea in females and hyposexuality, erectile dysfunction, or gynecomastia in males. Hyperprolactinemia was diagnosed by serum PRL greater than 324 $\mu\text{U}/\text{mL}$ in men and greater than 496 $\mu\text{U}/\text{mL}$ in women (nonpregnant). PRL was measured with the Elecsys Prolactin II electrochemiluminescence immunoassay (Cobas-Roche); and patients subsequently underwent magnetic resonance imaging scanning of the pituitary. The case-control-matched PA and essential hypertensive (EH) populations (1:3) have been described in detail elsewhere (24). Patients on medication that could cause hyperprolactinemia were excluded (25).

Cell culture

The adrenocortical carcinoma NCI H295R cell line were cultured as described (26) and incubated with 100 nM human prolactin (2500 $\mu\text{g}/\text{L}$; 53 000 $\mu\text{U}/\text{mL}$) recombinantly expressed in *Escherichia coli* (Sigma-Aldrich) for 48 hours after a 24-hour starvation period in 0.1% NuSerum (BD Biosciences). Aldosterone measurements were performed by a solid-phase RIA, ALDOCTK-2 (DiaSorin). The sensitivity of the assay is 5 pg/mL with cross-reactivities of 100% for aldosterone and less than 0.1% for 17-isoaldosterone and other steroids (27).

GH3 cell culture and transfection was performed as described (28) using the pA3PRL luciferase reporter construct (a kind gift from A. Gutierrez-Hartmann, University of Colorado, Denver, Colorado) (29). Cells were pretreated with the specific mineralocorticoid receptor (MR) antagonist eplerenone (10 μM ; Sigma-Aldrich) or vehicle for 1 hour and then treated with aldosterone (0–100 nM; Sigma-Aldrich) for 6 hours. Luciferase activity was determined using the Luciferase reporter assay (Promega) according to the manufacturer's instructions. Addition of 0.5 μg plasmid expressing the β -galactosidase gene ensured transfection efficiency control. Data are expressed as the ratio of relative luciferase to β -galactosidase activity. Each measurement was performed in triplicate.

Gene expression assays

Total RNA was extracted from tissues (normal adrenals, APA, normal kidneys, or prolactinomas) or from NCI H295R cells and reverse transcribed as described (26). Normal adrenals were obtained from normotensive individuals undergoing laparoscopic nephrectomy for localized renal carcinomas: in all cases, careful histological examination excluded the involvement of the adrenal in the tumor lesion. TaqMan real-time PCR was used to determine gene expression levels of *PRLR*, *CYP11B2*, *NR3C2*, or *HSD11B2* using *GAPDH* as the endogenous reference gene, and fold changes were determined using the $2^{-\Delta\Delta\text{Ct}}$ quantification method as described previously (26). In this study only a single reference gene was used, which could be a potential limitation of the study.

Statistical analyses

Data are shown as mean \pm SEM. Statistical analyses were calculated using SPSS statistics 17.0 software (SPSS Inc, 2009). Statistical significance between groups was calculated for normally distributed variables by an ANOVA using a Bonferroni post hoc analysis for multiple comparisons. For nonnormally distributed variables, a Kruskal-Wallis test was applied for multiple comparisons and a Mann-Whitney test for single comparisons. A probability of $P < .05$ was considered statistically significant.

Results

Description of cohorts

Coexisting prolactinoma-PA was diagnosed in 4 of 584 PA patients treated in the Division of Endocrinology, Ludwig-Maximilians-University (Munich) and in 3 of 442 PA patients of the Internal Medicine and Hypertension Unit, University of Turin, since 1990 and 1991, respectively. The prevalence of prolactinoma in the normal population is reported as 6–50 per 100 000 (5, 6); therefore, the incidence of prolactinoma in each PA cohort is increased 10- to 100-fold.

The clinical features and management of the Munich-Turin prolactinoma-PA case-series are shown in Table 1. The seven patients comprise five men and two women: all men were diagnosed with macroprolactinomas, one of the women with a microprolactinoma, and the remaining woman with idiopathic hyperprolactinemia. A notable feature of this case series was the predominance of macro- over microprolactinomas; interestingly, in a literature search of coexisting prolactinomas-PA, macroprolactinomas again predominated over microprolactinomas coexisting with PA with five of the seven cases comprising a macroadenoma (Supplemental Table 1).

Table 1. The Munich-Turin Prolactinoma-PA Case-Series: Clinical Features and Management

Patient	Sex	Age at Diag., y	Age Onset hyperprolactinemia, y + Symptoms	PRLoma Type + Size at Diag.	Therapy for PRLoma	PRL at Diag., $\mu\text{U/mL}^a$	Age, y	Age, Onset HT, y	Lowest Serum K^+ , mEq/L	AVS	PA Subtype	Aldosterone, ng/L, and Renin, mU/L, or PRA, ng/mL · h	Therapy for PA	Size of Adrenal Imaging
MU1	M	35	35 visual field defects	MacroPRLoma size unknown	Transsphenoidal surgery and radiotherapy	Unknown	55	53	3.2	Bilateral PA	BAH	PAC 249 PRA 0.08	Spironolactone	Normal
MU2	M	39	39 fatigue, infertility	MacroPRLoma 12 mm	Cabergoline, transsphenoidal resection	6508	39	29	3.7	Bilateral PA	BAH	PAC 143 PRC 3.2	Spironolactone	Normal
MU3	M	57	56 visual field defects	MacroPRLoma 35 mm	Quinagolid cabergoline	45 289	64	53	3.5	Not selective	BAH	PAC 341 PRC < 1	Left ADX + spironolactone	Left nodule 20 mm
MU4	M	63	61 hyposexuality	MacroPRLoma 20 mm	Cabergoline	23 946	63	42	3.1	Not performed	APA	PAC 444 PRC 1.2	Left ADX	Left nodule 46 mm
TO1	F	51	51 oligomenorrhea	Idiopathic hyperprolactinemia	Bromocriptine	840	51	51	3.8	Left PA	APA	PAC 450 PRA 0.1	Left ADX	Left nodule 10 mm
TO2	F	51	51 oligomenorrhea galactorrhea	MicroPRLoma 10 mm	Bromocriptine	1200	51	44	3.4	Left PA	APA	PAC 480 PRA 0.1	Left ADX	Left nodule 20 mm
TO3	M	63	61 hyposexuality	MacroPRLoma 30 mm	Cabergoline	3481	63	39	3.6	Left PA	APA	PAC 162	Refuses ADX	Left nodule

Patient	Sex	Age at Diag.	Age Onset hyperPRLemia, y + Symptoms	PRLoma Type + Size at Diag.	Therapy for PRLoma	PRL at Diag., $\mu\text{U}/\text{mL}$ ^a	Age, g-PA, y	Age, Onset HT, y	Lowest Serum K^+ , mEq/L	AVS	PA Subtype	Aldo, ng/L, and Renin, mU/L, or PRA, ng/mL · h	Therapy for PA	Size of Adrenal Imaging
			y									PRA 0.2		e 5 mm

Abbreviations: ADX, adrenalectomy; Aldo, aldosterone; AVS, adrenal venous sampling; diag., diagnosis; F, female; HT, hypertension; hyperPRLemia, hyperprolactinemia; K^+ , potassium; M, male; macroPRLoma, macroprolactinoma; microPRLoma, microprolactinoma; MU, Munich; PRA, plasma renin activity; PRC, plasma renin concentration; PRLoma, prolactinoma; TO, Turin.

^aHyperprolactinemia was diagnosed when serum prolactin was greater than 500 $\mu\text{U}/\text{mL}$ in Munich and greater than 324 $\mu\text{U}/\text{mL}$ in men and greater than 496 $\mu\text{U}/\text{mL}$ in women (nonpregnant) in Turin.

Only one patient displayed elevated PTH (patient MU4, Table 1) and was diagnosed with primary hyperparathyroidism. This patient was diagnosed with hyperprolactinemia first and with hyperparathyroidism 9 years later, although calcium levels were first elevated retrospectively in the same year as the diagnosis of hyperprolactinemia. Clinical symptoms of other MEN1-related tumors were absent in this patient and he refused genetic testing for MEN1. The APA from the two patients from Turin who underwent adrenalectomy were used for sequencing the *KCNJ5*, *ATP1A1*, *ATP2B3*, and *CACNA1D* genes for mutations causing an increase in aldosterone biosynthesis (30). Patient TO1 carried a *KCNJ5* G151R mutation, and patient TO2 was negative for all mutations in these four genes described to date. The two patients from the Munich center who underwent adrenalectomy were operated before this center began systematic collection of tumors in 2008.

There were five cases of hyperprolactinemia (no macroprolactinomas) in the general EH population of 14 790 patients compared with the 3 of 442 PA patients (one macroprolactinoma) recruited in the same center ($P = .001$, Supplemental Table 2). This represents a 20-fold increase in the PA cohort compared with the general EH population. The prevalence of hyperprolactinemia in the general EH population is consistent with that reported in the normal population of 6–50 cases per 100 000 (5, 6).

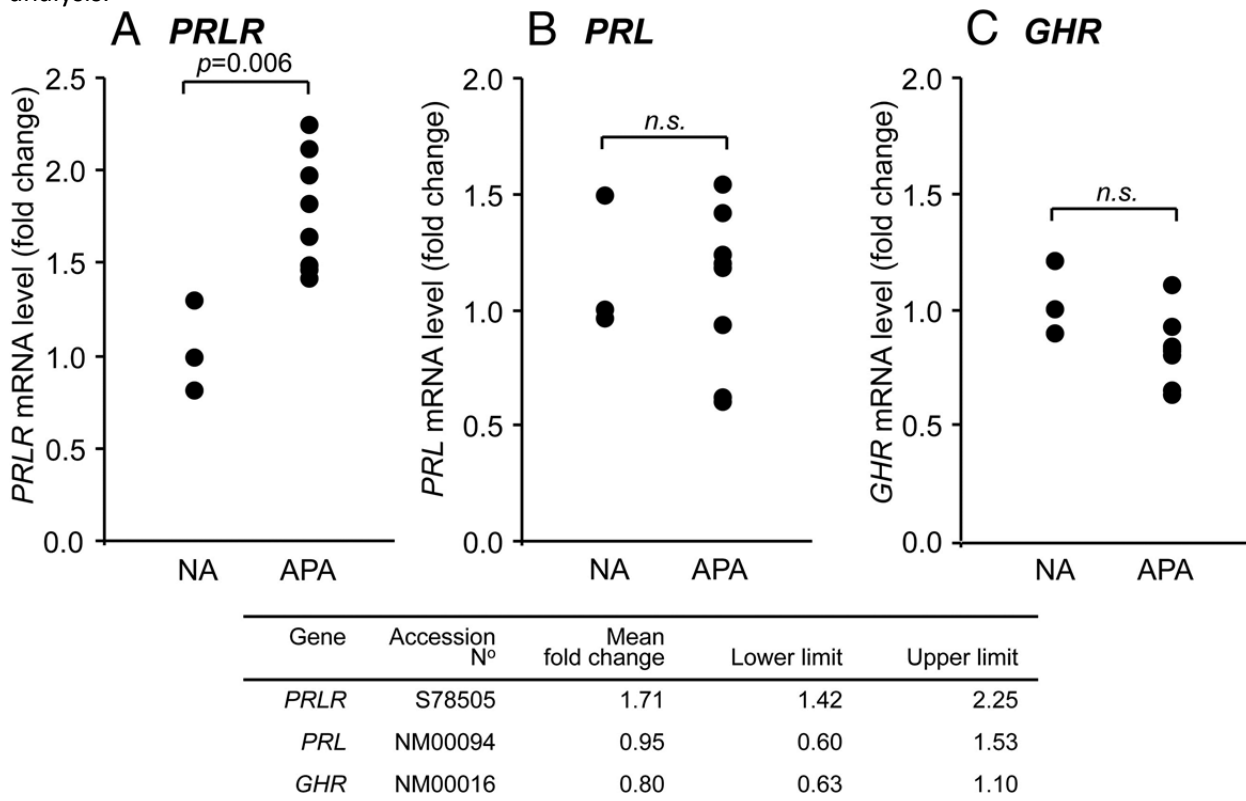
We also compared the number of cases of hyperprolactinemia in PA patients ($n = 270$) who were case-control matched 1:3 with EH patients (Supplemental Table 3). These two cohorts were recruited retrospectively and have been described in detail in a previous study (24). They were matched for sex, age, systolic and diastolic blood pressure at the first medical visit, duration of hypertension, body mass index, smoking habits, and comorbidity for type 2 diabetes mellitus. The 270 PA patients of the PA-EH matched cohort were part of the PA cohort ($n = 442$) used for comparison with the general EH cohort. There were two cases of hyperprolactinemia in the PA cohort ($n = 270$), and there were no cases of hyperprolactinemia in the case-control matched EH cohort ($n = 810$) (Supplemental Table 3).

The *PRLR* gene is up-regulated in APA compared with normal adrenals

A microarray study performed previously identified differentially expressed genes (defined in that study as an at least 2-fold difference in expression) between eight APA (from five women and three men) and three normal adrenals (26). In this study, we reanalyzed the microarray data for differences in expression of the *PRLR* between APA and normal adrenals. The *PRLR* was 1.7-fold up-regulated in APA ($P = .006$) (Figure 1). In contrast, the prolactin gene (*PRL*) and the GH receptor (*GHR*) gene, which belongs to the same receptor family as *PRLR*, showed no differences in expression between APA and normal adrenals. We validated the

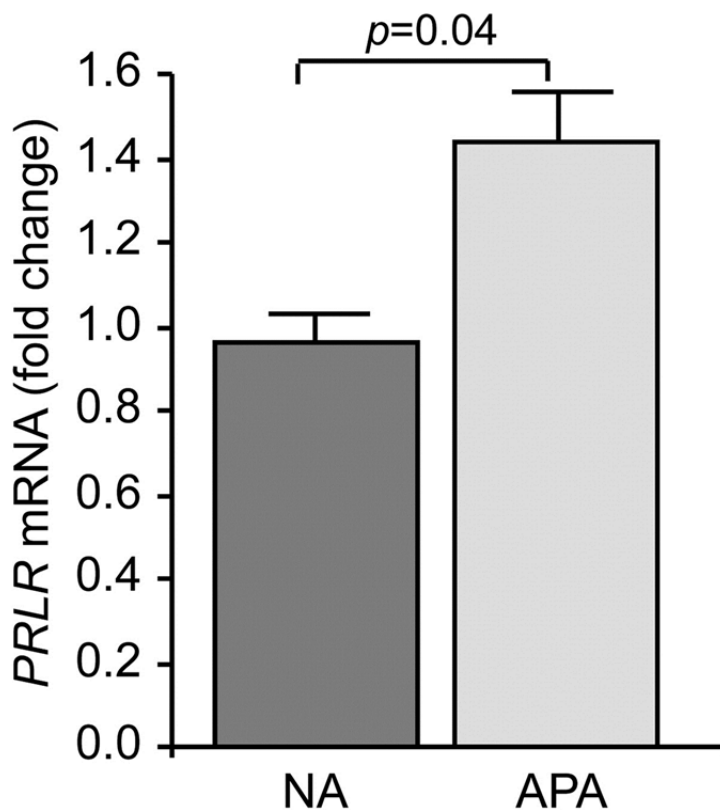
up-regulation of the *PRLR* in a broader set of adrenal samples (30 APA and nine normal adrenals) by real-time PCR. The APAs were removed from 15 women and 15 men. None of the APAs used for gene expression studies (microarray or real time PCR) were from patients with coexisting prolactinoma-PA. In the total sample set of APAs, the transcription of the *PRLR* gene was up-regulated 1.50-fold \pm 0.12-fold ($P = .04$) (Figure 2). There was no significant gender difference in the expression of the *PRLR* gene in APA compared with normal adrenals (1.50-fold \pm 0.16-fold up-regulation in women and 1.51-fold \pm 0.18-fold up-regulation in men).

Figure 1. *PRLR* gene up-regulation in APA compared with normal adrenals determined by microarray analysis.



Data show fold changes in mRNA levels of genes expressed in APAs ($n = 8$) compared with NA (normal adrenals; $n = 3$) from a microarray using an Affymetrix GeneChip HG-U133 Plus 2.0 gene expression profiling platform (26). A, *PRLR*, prolactin receptor gene; B, *PRL*, prolactin gene; C, *GHR*, growth hormone receptor gene. The bottom below the figure shows the means and the lower and upper limits of the fold changes in gene expression in the APA. n.s., not significant.

Figure 2. Validation of *PRLR* gene up-regulation in APA by real-time PCR.

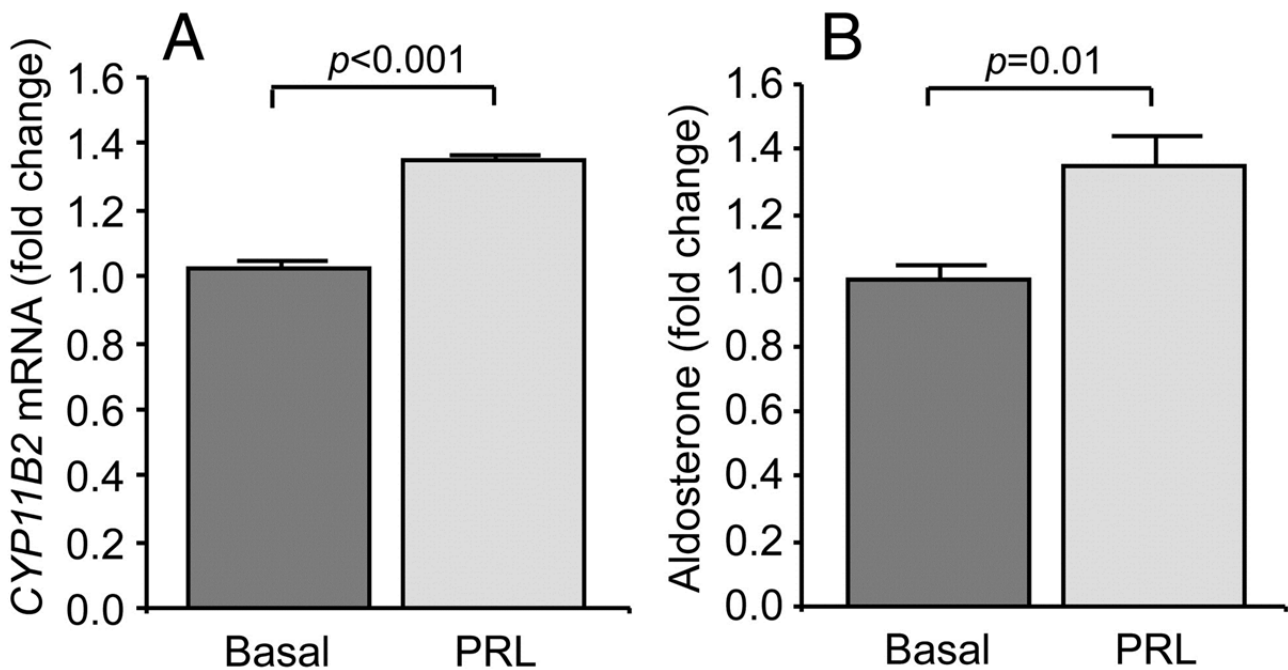


TaqMan gene expression assays were used in real-time PCR to determine fold changes ($2^{-\Delta\Delta Ct}$ quantification method) in *PRLR* mRNA levels in APAs ($n = 30$) compared with NA (normal adrenals, $n = 9$). *GAPDH* was used as the endogenous reference gene. Data shown are means \pm SEM.

PRL up-regulates *CYP11B2* gene expression and stimulates aldosterone production in adrenal cells

Incubation of NCI H295R cells with PRL (100 nM) had no effect on *PRLR* gene expression (fold changes over basal levels = 1.04 ± 1.03 and 1.16 ± 0.08 at 24 h and 48 h, respectively). In contrast, PRL (100 nM) resulted in a 1.32-fold \pm 0.02-fold up-regulation ($P < .001$) in *CYP11B2* gene expression (the gene encoding aldosterone synthase) and a 1.34-fold \pm 0.09-fold increase in aldosterone production ($P = .01$) (Figure 3, A and B, respectively). The concentration of PRL used to treat the H295R cells (100 nM) is equivalent to 53 000 $\mu\text{U}/\text{mL}$ and is within the range of the highest PRL concentration observed in one of the patients of our case series with a macroprolactinoma (Table 1, MU-3, 45 289 $\mu\text{U}/\text{mL}$). This concentration is 12-fold higher than during the third gestational period of pregnancy (209 ng/mL or 4430 $\mu\text{U}/\text{mL}$) and greater than 20-fold higher than during the fourth (2434 ± 409 $\mu\text{U}/\text{mL}$, mean of nine women) and eighth weeks of lactation (2253 ± 443 $\mu\text{U}/\text{mL}$, mean of 11 women) (31).

Figure 3. Effect of PRL on *CYP11B2* gene expression and aldosterone production in adrenal cells.

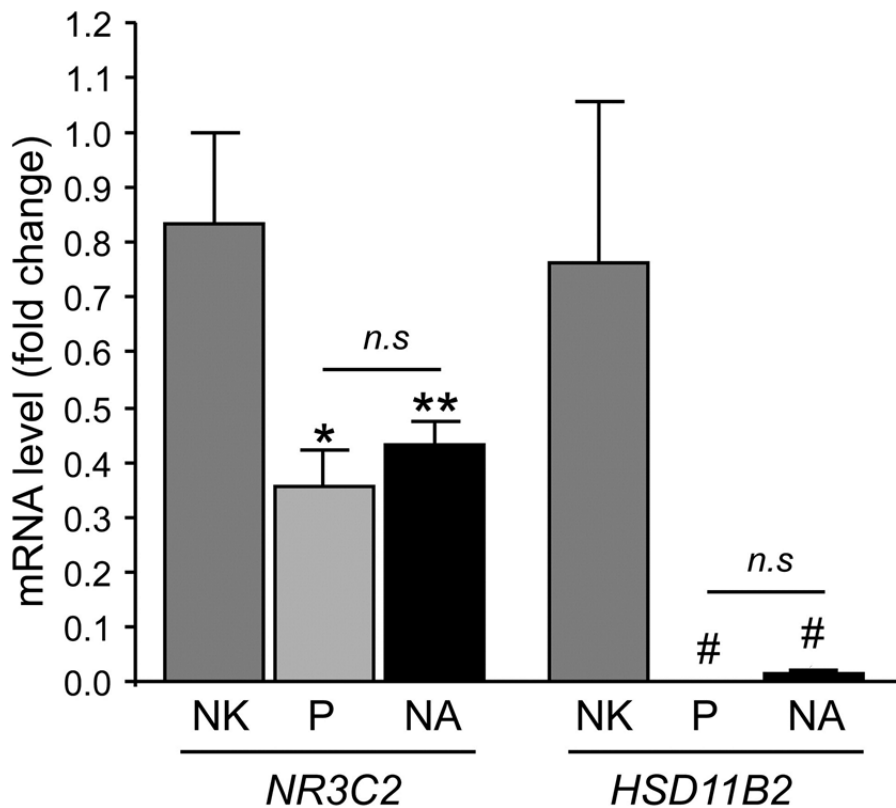


NCI H295R cells (1×10^6 cells/well) were grown in six-well plates for 24 hours in complete medium before incubation for a further 24 hours in 0.1% serum. After 48 hours of stimulation with 100 nM PRL in medium with 0.1% serum, total RNA was extracted and reverse transcribed, and *CYP11B2* gene expression was determined by TaqMan real-time PCR using *GAPDH* as the endogenous reference gene and the $2^{-\Delta\Delta Ct}$ method (A). Aldosterone production was measured in culture medium by RIA (Sorin Biomedical Diagnostics) (B). Data shown are the mean of eight experiments \pm SEM. *, $P < .001$; **, $P = .01$.

Prolactinomas express the mineralocorticoid receptor gene but not the gene encoding 11 β -hydroxysteroid dehydrogenase 2

The expression of the genes encoding the MR (*NR3C2*) and 11 β -hydroxysteroid dehydrogenase 2 (*HSD11B2*) were determined in prolactinomas (n = 6) and normal adrenals (n = 7) compared with normal kidneys (n = 3) by TaqMan gene expression assays in real-time PCR. *NR3C2* mRNA was present in all three sample sets. In contrast, *HSD11B2* was not detected in prolactinomas despite 45 cycles of amplification (Figure 4).

Figure 4. *NR3C2* and *HSD11B2* gene expression in prolactinomas and normal adrenals compared with normal kidneys.

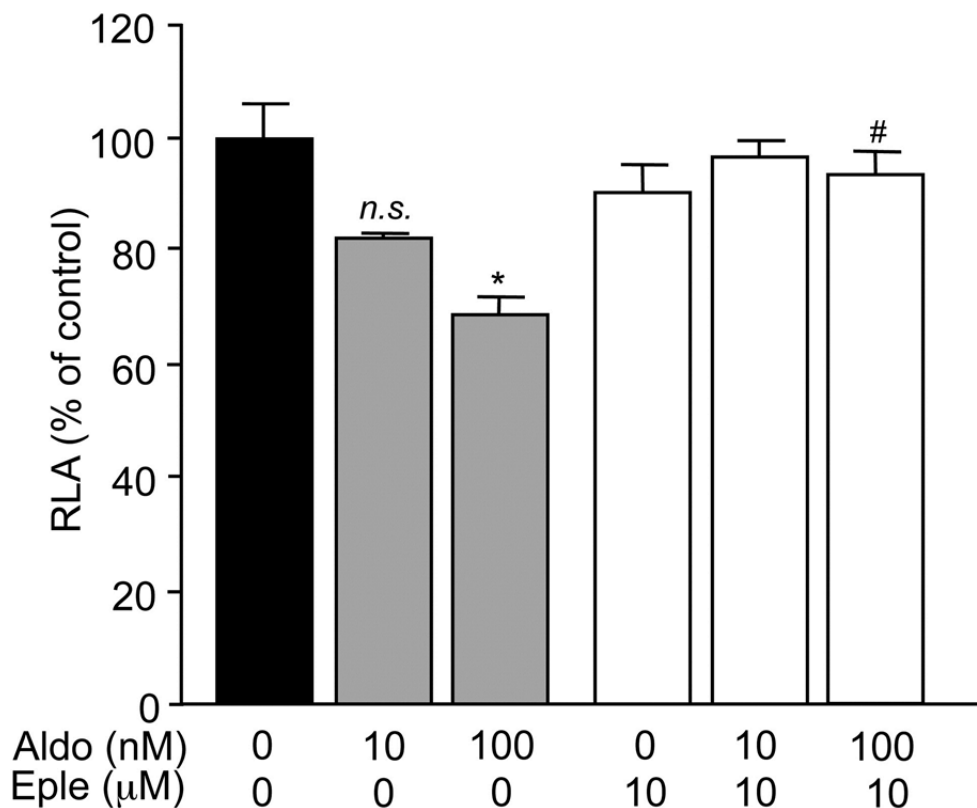


Data are shown as fold changes in mRNA levels of *NR3C2*, which encodes the MR, and *HSD11B2*, which encodes 11 β -hydroxysteroid dehydrogenase 2 in prolactinomas (n = 6) and normal adrenal tissues (n = 7) compared with normal kidneys (n = 3). TaqMan gene expression assays were used in real-time PCR using *GAPDH* as the endogenous reference gene and the $2^{-\Delta\Delta Ct}$ method to calculate fold changes in mRNA levels compared with the normal kidney sample with the median expression level. NA, normal adrenal; NK, normal kidney; n.s., not significant; P, prolactinoma. *, $P = .006$ vs NK; **, $P = .015$ vs NK; #, $P = .000$ vs NK.

Aldosterone does not increase PRL promoter activity in rat pituitary cells

Incubation of rat mammosomatotrophinoma GH3 cells transfected with a rat PRL-luciferase reporter gene with aldosterone (10 or 100 nM) did not induce luciferase gene expression, but rather an eplerenone-sensitive inhibitory effect was observed compared with vehicle-treated control cells (Figure 5). In our coexisting prolactinoma-PA case series, the plasma aldosterone concentration (PAC) range was from 143 to 480 pg/mL (Table 1) that corresponds to 0.4–1.3 nM aldosterone. The highest PAC value in the Munich PA cohort to date is 2130 pg/mL (5.8 nM) and in the Turin PA cohort, 1551 pg/mL (4.2 nM).

Figure 5. Effect of aldosterone on PRL promoter activity in rat pituitary cells.



Aldosterone dose response (0, 10, 100 nM) after 6 hours of treatment on PRL promoter activity in GH3 cells pretreated in the presence (white bars) or absence (gray bars) of eplerenone (10 μM) for 1 hour is shown. Treatments were done in serum-free, phenol red-free DMEM. The vehicle-only control (0.002% dimethylsulfoxide) is shown (black bar). Aldo, aldosterone; Eple, eplerenone; n.s., not significant; RLA, relative luciferase activity. Data are shown as means ± SEM from three measurements, presented as percentage of vehicle control. *, $P = .002$ vs vehicle; #, $P = .002$ vs aldosterone (100 nM).

Discussion

Herein we present seven cases of coexisting prolactinoma-PA recruited through two referral centers in Munich and Turin. Four of these cases were recruited from the Munich cohort comprising 584 PA patients, and three cases were from the Turin cohort comprising 442 PA patients. Because the prevalence of prolactinomas in the normal population is reported as 6–50 per 100 000, this represents a marked increased incidence of prolactinomas in two independent PA cohorts.

The Munich-Turin case series comprises five cases of macroprolactinoma-PA, which were all diagnosed in men, and two cases in women consisting of a microprolactinoma-PA and a case of idiopathic hyperprolactinemia-PA. Coexisting prolactinoma-PA has been reported previously, but a literature search revealed that this is a rarely reported condition. We found seven previously reported individual cases that also comprised five macroprolactinoma-PA (three women and two men) and two microprolactinoma-PA (both women), and the clinical features of these cases are summarized in Supplemental Table 1 (14–20). Because macroprolactinomas are defined on the basis of not just their size but also the associated high serum PRL level (9), the notable predominance of macroprolactinoma-PA in our case series as well as in the cases reported in the literature raises the hypothesis that elevated concentrations of PRL could play a role in the pathogenesis of PA. Given the difficulty of defining the temporal onset of PA and hyperprolactinemia, we cannot determine which endocrine condition came first but only hypothesize that hyperprolactinemia facilitated the clinical manifestation of PA. Therefore, to strengthen this hypothesis, we analyzed the prevalence of hyperprolactinemia in essential hypertensive cohorts. The prevalence of hyperprolactinemia was 20-fold higher in a PA cohort of 442 patients compared with a general EH cohort of 14 790 patients,

which also had no cases of macroprolactinoma. Furthermore, whereas there were two cases of hyperprolactinemia/prolactinoma in a PA cohort of 270 patients, there were no cases at all in a case-control matched 1:3 EH cohort comprising 810 patients.

Although reports of APAs coexisting with prolactinoma as part of the MEN1 syndrome have been described previously, the patients of our case series are clearly distinct from this syndrome. Importantly, none of our patients displayed family histories with MEN1 phenotypes, and furthermore, parathyroid and pancreatic tumors were absent in all patients with one exception, in a patient who displayed elevated PTH levels and who refused genetic testing. Several precedents exist for the association of PA with primary hyperparathyroidism in the absence of a genetic background of MEN1 (32–34). Therefore, considering the above, we reason that this patient does not have prolactinoma-PA as part of the MEN1 syndrome; however, in the absence of genetic testing of this patient, we cannot definitively exclude this possibility.

Immunohistochemical studies have demonstrated that the *PRLR* is expressed throughout all three zones of the adrenal cortex with an equal expression in the zona glomerulosa and the zona fasciculata and a possibly slightly increased expression in the reticularis. The medulla expresses very low levels, displaying weak staining on immunohistochemistry (35, 36). In fact, the adrenal gland and the uterus express the highest levels of the *PRLR* gene of the 45 human tissues analyzed in the GTEx Portal database2 (dbGaP accession number phs000424.vN.pN; www.gtexportal.org). This high level of *PRLR* expression is consistent with a physiological role of PRL in normal adrenal cell function. However, despite the high level of adrenal expression of the *PRLR*, we demonstrated by microarray analysis that the *PRLR* is up-regulated in APAs compared with normal adrenals, and this up-regulation was validated on a larger and independent set of adrenal samples by real-time PCR. The up-regulation of the *PRLR* in APAs may reflect a mechanism whereby the zona glomerulosa becomes sensitized to PRL and contribute to the hyperplasia of the adrenal gland observed in PA.

We demonstrate that a PRL concentration (100 nM, 53 000 μ U/mL) that can be found in patients with macroprolactinomas causes a significant increase in *CYP11B2* gene expression and aldosterone secretion in vitro. Interestingly, the level of increase of aldosterone production that we observed is highly similar to that described by Glasow et al (35) in a study in human primary adrenal cell cultures that used the same PRL concentration as in the present study.

Dopamine is one of the major regulators of PRL secretion that acts through the activation of the dopamine receptor D2 expressed at the surface of anterior pituitary lactotroph cells. Dopamine receptor D2 activation results in a reduction of PRL gene expression and exocytosis by a variety of intracellular signaling mechanisms (37). A link between dopaminergic tone, PRL, and the renin-angiotensin-aldosterone system has been demonstrated previously, and a reduction of dopaminergic tone has been hypothesized to provide a common pathway that connects increased prolactin and aldosterone production. In fact, mice homozygous for a disrupted dopamine D2 receptor (*D2*^{-/-} mice) display increased aldosterone production and hypertension that is markedly reduced by MR blockers (38).

Elevated levels of basal, angiotensin II-simulated, and ACTH-stimulated aldosterone were observed in patients with tumoral hyperprolactinemia on a high-sodium diet but not during sodium restriction (39). In another study, increased aldosterone levels were observed after angiotensin II administration in women with nontumoral hyperprolactinemia compared with healthy individuals (40). In both studies, the altered responses were normalized after the correction of hyperprolactinemia (39, 40).

The MR binds both glucocorticoids and mineralocorticoids with a similar high affinity (41). The specificity of the MR for aldosterone in target tissues is conferred by 11 β -hydroxysteroid dehydrogenase that converts cortisol to cortisone. To exclude the possibility of PA contributing to the pathophysiology of hyperprolactinemia, we demonstrated by real-time PCR that, although prolactinomas express the MR (*NR3C2*), the gene encoding 11 β -hydroxysteroid dehydrogenase 2 (*HSD11B2*) was undetectable. Thus,

because circulating levels of glucocorticoids are 1000 times higher than aldosterone in vivo, aldosterone cannot bind to the MR in prolactinomas. Furthermore, we showed that in the rat pituitary GH3 cell line, shown previously to express the MR (42), aldosterone did not activate the PRL promoter in a luciferase reporter assay but, rather, had an inhibitory effect. Because high supraphysiological concentrations caused this effect and because the pituitary does not appear to be a target of aldosterone action, the inhibitory effect we observed on the PRL promoter in vitro is probably not relevant in vivo. Therefore, it is highly unlikely that high aldosterone concentrations could be a contributory factor to the increased incidence of prolactinoma in coincident prolactinoma-PA.

In conclusion, this study provides evidence for a direct role of high levels of prolactin on the stimulation of aldosterone production, indicating a potential pathophysiological link between hyperprolactinemia/prolactinoma and the occurrence of PA in cases in which the two entities coexist. To clarify the potential link between these two endocrine conditions, it would be of interest to investigate the potential occurrence of PA in a hyperprolactinemia cohort or to examine aldosterone to renin ratios in patients with macroprolactinomas.

Abbreviations:

APA	aldosterone-producing adenoma
BAH	bilateral adrenal hyperplasia
EH	essential hypertensive
MEN1	multiple endocrine neoplasia type 1
MR	mineralocorticoid receptor
PA	primary aldosteronism
PAC	plasma aldosterone concentration
PRL	prolactin
PRLR	PRL receptor.

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