Primary Hyperparathyroidism With Concurrent Primary Aldosteronism

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Primary aldosteronism (PA) is a common cause of secondary hypertension, because it involves 11.2% of referred hypertensive patients.\(^1\) Primary hyperparathyroidism (PPTH) is much less common, with a prevalence that, albeit imprecisely known, is probably $<$0.01% in unselected hypertensives. However, arterial hypertension develops in the majority (56% to 80%) of the PPTH patients,\(^2\) which can explain why they are held to be at increased risk for cardiovascular complications and death.\(^3\) The association of PPTH with arterial hypertension and increased cardiovascular risk would appear to be paradoxical, inasmuch as the parathyroid hormone (PTH) has been described to induce vasodilation through endothelium-independent mechanisms.\(^4\) Therefore, it would be expected to lower rather than to raise blood pressure.\(^5\) The mechanisms by which excess PTH increases blood pressure remained obscure until Mazzocchi et al.\(^6\) reported that PTH stimulates in vitro the secretion of aldosterone from human adrenocortical cells in a concentration-dependent manner. These findings suggested that PTH acts as an aldosterone secretagogue that might be involved in causing human PA. However, whether this mechanism, although appealing, could be involved in causing human PA and might explain the development of arterial hypertension in patients with PPTH remained unsupported by any clinical data.

We herein report on a patient who presented with resistant arterial hypertension and was found to have PA. Unilateral adrenalectomy resulted in cure of the PA and control of blood pressure despite a tapering of antihypertensive treatment, but the patient developed hyperparathyroidism caused by a PTH-secreting adenoma that was surgically removed. Gene expression and immunohistochemistry studies unveiled the expression of type 1 PTH receptor in the aldosterone-producing adrenocortical nodules and of the mineralocorticoid receptor (MR) in the nuclei of parathyroid adenoma cells. This latter finding was confirmed in a series of normal human parathyroid glands. Thus, this unique case and the related findings support the notion that undetected hyperfunctioning of the parathyroid gland can contribute to maintaining hyperaldosteronism in PA. It also suggests the existence of a bidirectional link between the adrenocortical zona glomerulosa and the parathyroid gland, which can be relevant for the regulation of calcium metabolism and blood pressure.

**Case**

A 68-year–old man was referred for chest pain and resistant hypertension (Figure 1). The patient had a 10-year history of hypertension, which had become resistant to therapy with atenolol at 100 mg, amlodipine at 10 mg, doxazosin at 4 mg, potassium canrenoate at 25 mg, hydrochlorothiazide at 12.5 mg, and telmisartan at 80 mg daily. He had a family history of primary (essential) hypertension and a personal history of previous cigarette smoking, dyslipidemia, paroxysmal atrial fibrillation, and coronary artery disease that was treated with percutaneous transluminal coronary angioplasty and stenting 2 years before.

**Physical Examination**

On presentation, the blood pressure was 215/122 mm Hg with heart rate of 56 bpm; the body mass index was 28.1 kg/m\(^2\). Auscultation of the abdomen revealed no abdominal bruits. At funduscopy, a stage II Keith-Wagener-Barker retinopathy was found. A transthoracic echocardiogram showed a normal systolic function (left ventricular [LV] ejection fraction: 60%) with a concentric LV hypertrophy (LV mass index: 185 g/m\(^2\), 80.9 g/m\(^2\); relative wall thickness: 0.54). Ambulatory blood pressure monitoring confirmed the resistance of blood pressure to treatment and evidenced a “nondipper” profile (daytime: 139.8/87.6 mm Hg, night-time: 164.5/96.0 mm Hg; normal range: <128.3/82.2 and 110.4/70.3 for daytime and nighttime, respectively).

**Laboratory Values and Imaging Tests**

The patient had a persistent hypokalemia (3.1 mEq/L), notwithstanding the aforementioned treatment with telmisartan and potassium canrenoate. Total and ionized calcium were 2.44 and 1.22 mmol/L, respectively. Because of the

Received March 31, 2011; first decision April 22, 2011; revision accepted July 11, 2011.

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This paper was sent to Friedrich C. Luft, associate editor, for review by expert referees, editorial decision, and final disposition.

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**Hypertension** is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.111.173948
severity of hypertension and presence of target organ damage, the screening tests to identify a cause of secondary hypertension were performed without withdrawal of the antihypertensive treatment. Plasma aldosterone concentration was elevated (24.8 ng/dl at baseline, 21.2 ng/dL postcaptopril), and plasma renin activity was suppressed (0.4 ng/ml/h) at baseline and showed a paradoxical decrease postcaptopril (0.1 ng/mL/h); therefore, the aldosterone:renin ratio was markedly elevated both at baseline and postcaptopril (62 and 212 \[\text{ng/dL}/\text{ng/ml/h}\]; normal values: \[\text{1.19 to 1.29 mmol/L}\]; parathyroid hormone (PTH)=17 to 73 ng/L).

A computed tomography evidenced bilateral (right 20 mm diameter and left 30 mm diameter) adrenocortical nodules. Adrenal vein sampling, which was bilaterally selective,7 evidenced a slightly predominant aldosterone production on the left side (lateralization index: 1.69).8

**Treatment**

Because of the resistance of hypertension to treatment and because several patients without clear-cut lateralization and with bilateral adrenal hyperplasia can be cured by adrenalectomy,9 the patient was submitted to left laparoscopic adrenalectomy, which was performed uneventfully. Histology showed a 30-mm adrenocortical adenoma, consisting predominantly of “fasciculata-like” cells and several small micronodules in the surrounding cortex (Figure 2).

Follow-up evaluation 3 months postadrenalectomy showed blood pressure values consistently <130/80 mm Hg, notwithstanding the withdrawal of doxazosin and hydrochlorothiazide and the tapering of antihypertensive medications to atenolol at 50 mg, amloidipine at 10 mg, and telmisartan at 80 mg. A prominent reduction of LV end-diastolic diameter and LV mass index (from 185 to 151 g/m²) was observed. However, the patient developed overt hypercalcemia (total calcium: 2.92 mmol/L, normal range: 2.10 to 2.55 mmol/L; ionized calcium: 1.51 mmol/L, normal range: 1.19 to 1.29 mmol/L), which led to the discovery of PPTH (PTH: 240 ng/L; normal range: 17 to 73 ng/L) and of a right parathyroid adenoma at scintigraphy. The latter was surgically removed with full correction of the PPTH and further lowering of blood pressure. The search for multiple endocrine neoplasia mutation and familial isolated hyperparathyroidism gave a negative result.10 At 2.5 years of follow-up, the patient is in good health, his blood pressure is normal with the same 3 drugs, and serum ionized calcium is consistently normal. A mild elevation of PTH (120 ng/L) was corrected with 25(OH) vitamin D supplementation. At a repeated computed tomography of the upper abdomen, the size of the right adrenocortical node was unchanged.

**Case Summary**

The case typifies the common combination of unrecognized PA and resistant hypertension in an adult with evidence of hypertension-related target organ damage and a history of cardiovascular events. The correction of PA with adrenalectomy and the development of overt PPTH suggest the occurrence of a functional link between the adrenocortical zona glomerulosa and the parathyroid gland, which was totally unsuspected thus far. Our patient’s blood pressure responded dramatically to adrenalectomy and then responded further to parathyroidectomy. We present this case to initiate a discussion of the possible roles of PTH in triggering or maintaining the hyperaldosteronism and, conversely, of aldosterone in the regulation of calcium metabolism. The use of molecular and immunohistochemistry techniques allowed a
straightforward demonstration of the presence of PTH receptors in the aldosterone-producing adenoma (APA) tissue and in surrounding satellite nodules and of the MR in the parathyroid-secreting cells, both in the PTH-secreting adenoma and in the normal parathyroid gland. What is less clear is the relevance of subtle alterations in PTH secretion caused by vitamin D deficiency or renal insufficiency and the excessive aldosterone to the refractoriness of hypertension in the vast bulk of patients who exhibit some degree of resistance to antihypertensive therapy.

Resistant Hypertension
Resistance to pharmacological therapy is not an uncommon encounter in the management of hypertension and frustrates both physicians and patients who face the risks of uncontrolled blood pressure but are unable to achieve the blood pressure goals. In the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial, ≈30% were receiving ≥3 medications at year 5, and similar rates were found in the more recently published Ongoing Telmisartan Alone and in Combination With Ramipril Global Endpoint Trial. Resistance to pressure reduction is also common in patients with other comorbidities, such as LV hypertrophy: in the Losartan Intervention for Endpoint Reduction in Hypertension Study of patients with LV hypertrophy, ≈50% of patients had their blood pressure reduced to ≈140/90 mm Hg after 5 years of intensive antihypertensive treatment.

Unrecognized forms of secondary hypertension are common causes of truly resistant hypertension, and among these causes PA is one of the most important, because it can affect from 11.0% to 20.9% of the patients. The impossibility of withdrawing the antihypertensive drugs bedevils the undertaking of the tests that are necessary to identify PA, thus rendering the diagnosis most challenging.

Identification of PA in Patients With Resistant Hypertension
According to the Endocrine Society Guidelines, certain categories of hypertensive subjects should be screened for PA by measuring plasma aldosterone and renin and then calculating the aldosterone:renin ratio, which allows for pinpointing cases with presumed PA. The screening tests must be, by definition, highly sensitive, and, therefore, they carry many false-positive cases. Hence, case detection should be followed by an exclusion test, with the purpose of demonstrating that the excess aldosterone secretion is autonomous from
the renin-angiotensin system. These tests include the oral sodium loading test, the saline infusion test, the captopril challenge tests, and the fludrocortisone with salt loading test. Unfortunately, the premise on which these tests are based does not often hold true, because aldosterone secretion is angiotensin dependent in most cases of idiopathic hyperaldosteronism and even in many cases of APA. Relying on these tests can, thus, lead to missing several curable APAs that show suppressible aldosterone excess after blunting renin. Moreover, because of the confounding effect of many antihypertensive drugs on the renin-angiotensin-aldosterone system, such strategy is unfeasible in patients with resistant hypertension. Under these circumstances, only some clues as to whether PA might be present can be gained by interpreting the renin, aldosterone, and aldosterone:renin ratio values based on knowledge of the effects of each class of drugs on these indices, as discussed elsewhere.

At our institution, we propose adrenal vein sampling to patients with resistant hypertension who have some clues to the presence of PA with the aim of identifying lateralized aldosterone excess. In the case herein described, this strategy allowed for resolving the resistance of blood pressure to drug treatment.

Mechanisms Driving Hyperaldosteronism in PA

In the presence of a normal-to-high level (>6.3 g/d) of NaCl, chronic hyperaldosteronism induces sodium and water retention and arterial hypertension, which suppress the renin-angiotensin system and, therefore, would blunt aldosterone secretion. Moreover, hypokalemia that accompanies many cases of PA would also be expected to lower aldosterone secretion. Therefore, the mechanisms underlying persistent aldosterone over secretion despite suppression of the renin-angiotensin system and the hypokalemia have been a “puzzle” for endocrinologists, and a quest for the triggering factor(s) has been ongoing for decades. Recently, a couple of mutations in the KCNJ5 potassium channel gene that are located in the selectivity filter were described by Choi et al. This would result in cell depolarization, opening of T-type Ca channels with ensuing activation of cell proliferation, and aldosterone secretion. However, these mutations were found in only approximately one third of large-sized APAs and, therefore, do not provide a mechanistic explanation for all cases of PA. Based on observation of this unique case and on previous in vitro studies, we put forward the hypothesis that the excess PTH could entail one such mechanism. We, therefore, undertook the experiments herein described to support this view.

Immunohistochemistry

Samples from the cortical adrenal adenoma, the normal parathyroid glands, and a normal kidney were formalin fixed and paraffin embedded and investigated for the presence of the MR as described in the online Data Supplement (please see http://hyper.ahajournals.org). To further confirm that the PTH-secreting cells were those expressing the MR, we similarly performed double immunocytochemistry using the antibody for the MR and either PTH or calcitonin on sections of normal human parathyroid glands.

Gene Expression Analysis of MR and PTH Receptor

The RNA from index case parathyroid and adrenal adenomas and from normal parathyroid (n=3), kidney (n=1), and adrenal cortex specimens was extracted. RNA (500 ng) was DNase treated (Ambion, Milan, Italy) and reverse-transcribed with Iscript (Bio-Rad, Milan, Italy) in a final volume of 20 μL. The MR and PTH receptor expression was measured by real-time RT-PCR with Universal ProbeLibrary Probes (Roche, Monza, Italy) in the LightCycler 480 Instrument (Roche, Monza, Italy).

MR Expression in Nuclei and Cytosol

The expression and intracellular localization of MR were examined by immunoblot analysis. To this aim, protein fractions from nuclei and cytosol were separated by using the NE-PER nuclear and amp kit (Pierce, Milan, Italy), as described in the online Data Supplement.

Results

The immunohistochemistry and gene expression studies evidenced the expression of the PTH receptor in the cytoplasm of the aldosterone-producing adenoma cells (Figure 2D, 2E, and 2G), thus documenting that PTH could directly influence the synthesis of aldosterone.

A similar combined approach documented the expression of the MR transcript in the parathyroid adenoma tissue (Figure S1E and S1F, available in the online Data Supplement) and of the MR protein in human parathyroid cells (Figure S1A, S1B, and S1D). Of note, whereas in the kidney tubules, the receptor was mostly localized in the cytoplasm, in the parathyroid tissue sections it was predominantly found in the nuclei. The immunoblotting on protein extracted from the cytosol and nuclear fraction (Figure S1D) confirmed these immunohistochemistry findings. Collectively, these results suggest binding of aldosterone to the MR with ensuing translocation to the nucleus.

The double immunocytochemistry on sections of normal human parathyroid glands using the antibody for the MR (stained in brown) and either PTH or calcitonin demonstrated a brown nuclear staining of the PTH-secreting cells (Figure S2A). A stronger nuclear and a weaker cytoplasm brown staining for the MR was evident also when the antibody against PTH was replaced by that specific for calcitonin (Figure S2B).

Discussion

Because PA can render hypertension refractory to medical therapy, the US Endocrine Society guidelines recommend the case detection of PA in patients with drug-resistant hypertension. In this patient, the normalization of blood pressure with treatment after unilateral adrenalectomy, which corrected the hyperaldosteronism, provides evidence for a causative role of the hyperaldosteronism in rendering blood pressure refractory to antihypertensive drugs.

PA is held to be autonomous from the renin-angiotensin system, but the term “primary” only denotes our scant knowledge of the mechanisms underlying the hyperaldosteronism. This case is, therefore, of interest from several perspectives.
standpoints, because it highlights one possible such mechanism and also suggests a bifunctional link between the parathyroid gland and the adrenocortical zona glomerulosa. The observation of bilateral macronodular hyperplasia, along with the finding of multiple adrenocortical nodules in the excised adrenal, collectively suggested that a systemically acting factor chronically stimulated the adrenocortical zona glomerulosa in both adrenal glands to overproduce aldosterone and to develop into hyperplasia, eventually resulting into micronodular and macronodular changes (Figure 2A through 2C).

Accordingly, in the excised adrenal cortex, we could show that not only the adenoma but also the nodules of hyperplasia expressed the type 1 PTH receptor at both the mRNA and the protein level (Figure 2D, 2E, and 2G). A similar PTH receptor expression was consistently found in other cases of PA because of APA from our tissue bank (data not shown) that did not show overt PPTH but only a subtle elevation of PTH levels. These findings likely explain why human dispersed adrenocortical cells responded with an aldosterone and cortisol release to either PTH or the PTH-related peptide.6 They also make unlikely that the action of PTH occurred via the MC2R (adrenocorticotrophic hormone) receptor, as suggested by others.6,26

The lack of overt hypercalcemia before adenectomy, when our patient had uncorrected PA, was also of peculiar interest. In fact, it could suggest not only that the hyperaldosteronism by causing hypercalciuria and lowering serum-ionized calcium27 can mask the PPTH but also that aldosterone and cortisol may exert a negative feedback on PTH release.

Consistent with this contention, we could document that the normal human parathyroid gland (Figure S1A and S1B) and excised parathyroid adenoma cells from patients with PPTH (Figure S1E) expressed the MR. Of note, at variance with the renal tubuli, a known tissue target of aldosterone, where the MR was predominantly found in the cytoplasm (Figure S1C), in these tissues the MR was predominantly found in the nuclei (Figure S1A and S1B). This finding was confirmed with immunoblotting on nuclear and cytosol protein fractions (Figure S1D), as well as at the mRNA level in the parathyroid adenoma of the index case (Figure S1E and S1F). A similar predominantly nuclear localization of the MR has been found recently in other tissues, including the heart,28,29 that are now regarded as targets of aldosterone action.

The steroid receptors reside predominantly in the cytoplasm and on ligand binding undergo a conformational change that exposes nuclear localization signals, which allow translocation to the nucleus.30 Accordingly, the predominant nuclear localization of the MR in the parathyroid cells of patients without PA (Figure S1A, S1B, and S1D) suggests a role also of normal plasma levels of aldosterone in tonically regulating PTH secretion (Figure S3).

It has to be reckoned that removal of the parathyroid adenoma was followed in our patient by correction of the PPTH and also by a further decrease of blood pressure. This is consistent with our observation that serum PTH levels were found to be directly related to systolic blood pressure in a large cohort of patients with PA (unpublished observations). It also suggests that PTH could play a role in causing the stiffening of large arteries, as suggested recently.31

Conclusions and Perspectives

While the PTH-related peptide (PTH-rP), a mediator of cancer hypercalcemia that also acts on type 1 PTH receptor, was reported to exert a proliferogenic effect in human adrenocortical carcinoma cells,32 there are at present no data on whether chronic hyperparathyroidism can exert a proliferogenic effect of the human adrenocortical zona glomerulosa. Hence, this issue deserves specific research efforts. In this case, although there was no regression of the adrenocortical nodule in the residual adrenal gland after parathyroidectomy, no further enlargement of the lesion was noticed at follow-up. Whether in the long-term the correction of PPTH will also cause regression of the macronodular and micronodular changes in the right adrenal gland remains to be established.

In summary, the report of unique clinical cases like this one is of importance, because it can lead to identification of previously unsuspected pathophysiological links, thus expanding our understanding of the pathophysiology of common causes of human hypertension as PA. In this regard, we have established a Web site (http://147.162.241.48) for investigators to report such unique cases, with the hope that the collection of these cohorts can provide a basis for further research.

Sources of Funding

This study was supported by research grants from the Foundation for Advanced Research in Hypertension and Cardiovascular Diseases (F.O.R.I.C.A.), the Società Italiana dell’Ipertensione Arteriosa, the University of Padua “Research Projects Program,” and the International PhD Program in Arterial Hypertension and Vascular Biology of the University of Padova.

Disclosures

None.

References


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Hypertension. 2011;58:341-346; originally published online August 8, 2011;
doi: 10.1161/HYPERTENSIONAHA.111.173948

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/58/3/341

Data Supplement (unedited) at:
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Primary Hyperparathyroidism With Concurrent Primary Aldosteronism

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Running title: Primary Aldosteronism with Hyperparathyroidism

Words count: body of text with references 4428, references: 32 Figures 2; Tables none.

Conflict of interest and financial disclosure to be disclosed: none.

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Methods

Immunocytochemistry

Samples from the cortical adrenal adenoma, the normal parathyroid glands, and a normal kidney were formalin-fixed and paraffin-embedded. Four μm-thick serial sections from the paraffin blocks were used for immunohistochemistry (IHC) with an indirect immunoperoxidase-based technique (Bond polymer Refine detection, Vision Biosystem, Newcastle upon Tyne, UK) and a fully automated system (Bond-maX, Vision Biosystem). The sections were dewaxed and rehydrated with Bond Dewax solution (Vision Biosystem), ethanol and distilled water. Antigen was retrieved by heating the sections previously immersed in Bond Epitope Retrieval solution 1 (Vision Biosystem) at 100 °C for 30 minutes. Endogenous peroxidase was blocked by treatment with 3% hydrogen peroxide and then incubated with a mouse monoclonal antibody against human MR (clone 1D5, produced in Dr. Gomez-Sanchez’s laboratory, 1:100 dilution) for 30 minutes. It was then detected by incubation with a secondary antibody labeled with horseradish peroxidase (HRP) and diaminobenzidine. The sections were counterstained with hematoxylin, dehydrated, cleared and mounted.

Gene expression analysis of MR and PTH receptor

The RNA from index case parathyroid and adrenal adenomas and from normal parathyroid (n=3), kidney (n=1) and adrenal cortex specimen was extracted. 500 ng of RNA was DNase treated (Ambion, Milano, Italy) and reverse-transcribed with Iscript™ (Bio-Rad, Milan, IT) in a final volume of 20 μL. The MR and PTH receptor expression was measured by real time RT-PCR with Universal ProbeLibrary Probes (Roche, Monza, IT) in the LightCycler 480 Instrument (Roche, Monza, IT). To avoid DNA amplification, PCR primers were designed to span exon sequences. Moreover, to verify their RNA specificity, we amplified non reverse-transcribed RNA as negative control. Two reference genes were used as positive controls, beta actin with parathyroid samples and porphobilinogen deminase (PBGD) with adrenal samples. PCR products were further analyzed with gel electrophoresis and amplicons were detected by the QuantityOne Program of VersaDOC 1000 (Bio-Rad, Milan, IT).

Mineralocorticoid Receptor expression in nuclei and cytosol

The expression and intracellular localization of MR was examined by immuno blot analysis in protein fractions from nuclei and cytosol that were separated by using the NE-PER® nuclear & amp kit (Pierce, Milan Italy) as described in the supplemental data (please see http://hyperahajournals.org). Briefly, parathyroid (n=2) and kidney (n=1) specimen were homogenized in lysis buffer with a Dounce homogenizer, following kit protocol. Protein concentration was determined with microBCA (Pierce, Milan, Italy), using bovine serum albumin standard. Lysate fraction (50 μg) was solubilized in Laemmli buffer and separated by electrophoresis through a polyacrylamide gel (7%). The proteins separated from the gel were electro blotted onto nitrocellulose membrane (Hybond ECL Amersham Biosciences Europe, Freeburg, Germany). The membranes were blocked 2 h in T-PBS and
thereafter incubated overnight at 4°C with a primary monoclonal antibody against MR (1:100 dilution), from clone 1D5, produced in Dr. Gomez-Sanchez’s laboratory. Detection was made with the Enhanced Chemiluminescence System (ECL) from Pierce (Milan, IT). Blots were analyzed by the QuantityOne Program of VersaDOC 1000 (Bio-Rad, Milan, IT).

References


Supplemental Figures

Fig. S1

Supplemental Figures

A 50 µm

B 20 µm

C 500 µm

D

Case 1 | Case 2
---|---
C | N
C | N

107 Kd

E

Index Case
Parathyroid
Untr. RNA
Negative control

131 bp

F

Fluorescence

Cycles

B actin
MR
Negative controls

Fig. S1
**Fig. S1:** Panel A-B: Representative sections of human parathyroid gland incubated with a specific antibody against the human mineralocorticoid receptor (MR) showing intense nuclear staining and a weaker cytoplasm staining. Panel C: A section of normal human kidney was similarly exposed to the same antibody as a positive control shows a strong cytoplasmic expression in the tubules and the lack of staining in glomeruli. No staining was observed in the parathyroid gland sections after omission of the primary antibody (not shown). Panel D: Immuno-blotting on nuclei and cytosol fractions of human parathyroid glands (n=2, Case 1 and 2)) showed a predominant expression of the MR protein in the nuclei. N= nucleus, C= cytosol.

Panel E: Agarose gel electrophoresis of MR gene amplicon from the parathyroid adenoma of index case (lane 2) and a normal human parathyroid (lane 3) shows the transcript of this gene in these tissues. Untranscribed RNA from normal human parathyroid (lane 4) and water served as negative controls (lane 5). Real time RT-PCR amplification curve (panel F) and melting curve analysis (not shown) confirmed the expression of the MR gene in the human parathyroid adenoma of index case. Beta actin gene was used as reference.

**Fig. S2:** Double immunocytochemistry on sections of normal human parathyroid gland with an antibody for the MR (in brown) and for either PTH (panel A) or Calcitonin (panel B) (both stained in red) showed that the MR was detectable mostly in the nuclei (stained in brown in both panels) and more weakly in the cytoplasm. The concomitant red staining of the cytoplasm demonstrated the localization of the MR in the PTH-secreting cells (panel A). The lack of staining for calcitonin (panel B) in the cells expressing the MR in both nuclei and cytoplasm is consistent with the lack of expression of this peptide in the parathyroid gland.
Fig. S3: Schematic representation of the possible bifunctional link between the adrenocortical zona glomerulosa and the parathyroid gland. Given the secretagogue effect of PTH on aldosterone, excess secretion of PTH can cause hyperaldosteronism, which in turn can modulate the secretion of PTH. Excess levels of PTH and aldosterone can have a detrimental effect on the vascular wall, resulting in vascular damage.