CASE REPORT

Familial adrenocorticotropin-independent macronodular adrenal hyperplasia with aberrant serotonin and vasopressin adrenal receptors

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Abstract

ACTH-independent macronodular adrenocortical hyperplasia (AIMAH) is rare and generally presents as a sporadic disease. We describe a familial case of AIMAH with *in vivo* and *in vitro* demonstration of aberrant 5-HT₄ and vasopressin adrenal receptors. Two sisters presented with clinical and biological features of mild Cushing's syndrome with bilateral macronodular adrenal enlargement on computerized tomography (CT)-scan evaluation. *In vivo* pharmacological tests showed a significant increase in plasma cortisol after terlipressin and metoclopramide administration. Unilateral adrenalectomy was performed in one of these patients. Reverse transcriptase-PCR analysis of the hyperplastic tissue revealed expression of 5-HT₄ receptor isoforms (a), (b), (c), (i), and (n), and of vasopressin receptors, V1 and V2. Their father and brother were overweight, had easy bruisability and presented with biological features of subclinical Cushing's syndrome. CT scan showed moderate adrenal enlargement. *In vivo* pharmacological screening tests for the detection of adrenal aberrant receptors in the brother were negative. Finally, three out of the two sisters' children were evaluated. They had neither clinical nor biological features of Cushing's syndrome. Their adrenal glands were normal on CT-scan evaluation. *In vivo* evaluation for the detection of aberrant adrenocortical receptors performed in one of these subjects was negative. In conclusion, this study shows that (i) familial AIMAH could be an autosomal dominantly inherited disorder; (ii) aberrant 5-HT₄ serotonin and vasopressin receptors can be expressed in familial AIMAH; and (iii) phenotypic expression of familial AIMAH could be varied in a same family and more pronounced in female than in male patients.

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Introduction

In adrenocorticotropin (ACTH)-independent Cushing's syndrome due to bilateral macronodular adrenal hyperplasia (AIMAH), cortisol production can be controlled by adrenal aberrant receptors, like gastro-intestinal peptide (GIP), β -adrenergic, luteinizing hormone/human chorionic gonadotropin (LH/hCG), vasopressin V1 or 5-HT₄ serotonergic receptors (review in (1)). Some of these receptors are physiologically absent in the adrenal cortex, like the GIP (2, 3) and the β -adrenergic receptors (4). Others like the LH/hCG receptor are physiologically present in the adrenal cortex, but are located normally only in the zona reticularis and therefore have no physiological role in cortisol production (5, 6). Finally, some receptors are normally expressed by the zona fasciculata of the

adrenal cortex, like vasopressin V1 (7–9) and 5-HT₄ serotonergic receptors (10), where they mediate the stimulatory effects of arginine vasopressin and 5-HT on cortisol production but to date their physiological role in the control of glucocorticoid secretion is unknown.

The more frequently expressed receptors in AIMAH are the serotonin 5-HT₄ and the vasopressin V1 receptors (11, 12). The serotonin 5-HT₄ receptor gene generates ten 5-HT₄ receptor splicing isoforms (a–i and n) which can be activated by metoclopramide and cisapride (13–17). Studies have shown that most 5-HT₄ receptor agonist-responsive AIMAH tissues overexpress the 5-HT₄ receptor (17, 18). However, the mechanism underlying the abnormal cortisol response to 5-HT₄ receptor agonists remains unclear in some AIMAHs (17). The actions of vasopressin are mediated through three different

membrane receptor types, V1, V2, and V3. In most patients with vasopressin-responsive AIMAH, cortisol secretion is regulated by the nonmutated V1-vasopressin receptor expressed either at higher or at similar levels in the adrenal tissues of AIMAH when compared with normal adrenals (19). Ectopic expression of V2 and/or V3 vasopressin receptors can also be involved in the regulation of cortisol production of vasopressin-responsive AIMAH (20).

Very few familial cases of AIMAH have been described (20–24). To date, the presence of aberrant receptors has been studied *in vivo* and *in vitro* in two families and only two members of each family were evaluated (20, 24). These previously reported patients with familial AIMAH express LH/hCG and/or vasopressin adrenal receptors. We report a familial case of AIMAH with aberrant expression of serotonin and vasopressin receptors.

Patients and methods

Patients

Seven members of the same family were investigated after informed consent of the patients (Fig. 1). *In vivo* screening for the detection of adrenal aberrant receptors was performed in four patients (patients III1, III2, III3, and IV4).

Patient II2, an 81-year-old man, suffered from type 2 diabetes and hypertension. He was overweight with a centripetal distribution of body fat mass, and presented with dorso-cervical fat pads and easy bruising.

Patient III1, a 57-year-old man, was overweight and had easy bruising.

Patient III2, a 56-year-old woman, suffered from hypertension and glucose intolerance. She had centripetal obesity, plethora, dorso-cervical and supraclavicular fat pads, and mild hirsutism. Her bone density was normal.

Patient III3, a 54-year-old woman, was overweight with a centripetal distribution of body fat mass. She was suffering from high blood pressure and type 2 diabetes mellitus. She had facial plethora and easy bruising. Bone density was normal.

Patient IV3, a 38-year-old man, was obese, and had supraclavicular fat pads and hypertension.

Patient IV4, a 35-year-old woman, had no clinical features of Cushing's syndrome.

Patient IV5, a 34-year-old man, was obese but had no specific clinical features of Cushing's syndrome.

Methods

Plasma cortisol was measured using a competitive immunochemiluminometric assay (ACS-Centaur Cortisol, intra-assay coefficient of variation (CV)=2.9%, interassay CV=3%; detection limit=5.5 nmol/l; normal range=118.6–618 nmol/l at 0800 h) provided by Bayer Diagnostics (Puteaux, France). Plasma ACTH was measured using an IRMA (Immunoassay Nichols Advantage; intra-assay CV=2.7%; interassay CV=8.4%; detection limit=0.22 pmol/l; normal range=2–11 pmol/l at 0800 h) provided by

Figure 1 Family's pedigree.

Nichols Institute Diagnostics (San Clemente, CA, USA). Urinary free cortisol was measured using an IRMA (Cortisol RIA kit) provided by Immunotech Beckman Coulter Company (Roissy, France); detection limit = 10 nmol/l; intra-assay CV = 5.8% and interassay CV = 9.2%; normal range = 38–208 nmol/day.

Diagnosis of Cushing's syndrome

The degree of cortisol excess was based on clinical symptoms and results of hormonal investigations. Overt Cushing's syndrome was characterized by the presence of specific clinical symptoms of hypercorticism, increased urinary free cortisol (> 208 nmol/day), mid-night plasma cortisol above 138 nmol/l, serum cortisol levels not suppressible below 50 nmol/l, and urinary free cortisol not suppressible below 28 nmol/day by 2 mg dexamethasone. Mild Cushing's syndrome was characterized by the presence of clinical symptoms of hypercorticism, increased midnight cortisol, and abnormal response under 2 mg dexamethasone in spite of normal urinary free cortisol. Subclinical Cushing's syndrome was defined as the presence of nonspecific clinical symptoms (obesity, hypertension, diabetes mellitus, etc.) and at least two abnormalities in hypothalamic–pituitary–adrenal axis function in the following: midnight plasma cortisol above 138 nmol/l, serum cortisol levels not suppressible below 50 nmol/l by 1 mg dexamethasone, serum cortisol levels not suppressible below 50 nmol/l by 2 mg dexamethasone, or urinary free cortisol not suppressible below 28 nmol/day, ACTH below 3.3 pmol/l, whereas urinary free cortisol level was in the normal range (25–31).

In vivo screening for aberrant adrenal receptors

The protocol used to detect *in vivo* adrenal aberrant receptors was modified from that described by Lacroix *et al.* (1). On the first day, plasma samples were taken in supine posture and then in upright posture. This was followed by a mixed meal test. Subsequently, the patient was injected with 250 µg tetracosactide (Synacthene; Novartis Pharma, Rueil-Malmaison, France). On the second day, a luteinizing hormone-releasing hormone (LHRH) test was performed (100 µg LHRH, Stimu-LH; Roussel Diamant, Paris-La Défense, France), followed by a thyrotrophin-releasing hormone (TRH) test (200 µg TRH, Stimu-thyroid-stimulating hormone; Roussel Diamant, Paris-La Défense, France). On the third day, plasma cortisol and ACTH responses to 1 mg glucagon IV (Glucagen; Novo Nordisk, Boulogne-Billancourt, France), 10 IU terlipressine IV (Glypressine; Ferring, Gentilly, France), 10 mg metoclopramide orally (Primperan; Sanofi-Synthelabo, Paris, France), or 10 mg cisapride orally (Prepulsid; Janssen-Cilag, Issy-les-Moulineaux, France) were studied. When a response to terlipressin

was observed, a desmopressin test (dDAVP 2.5 µg SC) was subsequently performed (1). The patients received 2 mg/day dexamethasone (0.5 mg orally every 6 h) throughout the study. *In vivo* screening tests for the detection of aberrant receptors were performed twice in patients III2 and III3 at a 3-year interval.

All the results of the tests were expressed as a percentage of increase from basal values, calculated as follows: (peak cortisol–basal cortisol)/basal cortisol × 100. Plasma cortisol increase was considered significant, when above 50% of the basal level (1).

In vitro detection of aberrant adrenal receptors

Tissue collection Adrenal hyperplasia tissue from patient III3 was obtained at surgery and immediately dissected by the pathologist. Hyperplasia explants were stored at –80 °C until RT-PCR experiments. Informed consent was obtained from the subject.

RNA extraction and real-time RT-PCR Total RNA from hyperplasia tissue was extracted by the acid guanidium–thiocyanate–phenol–chloroform procedure using Tri Reagent (Sigma). The concentration of total RNA was determined by measuring the optical density at 260 nm. Real-time RT-PCR analysis was carried out as described previously (32) in order to quantify 5-HT₄ receptor mRNA in the hyperplastic tissue. The primers and fluorogenic TaqMan probe used for these experiments hybridized to all 5-HT₄ receptor splice variants (17). Briefly, 1 µg total RNA from the tissue was converted to single-stranded cDNA using SuperScript II from Life Technologies with oligo (dT)_{12–18} primer (0.5 µg/ml), and the cDNA was diluted and aliquoted into microtiter plates. For each 25 µl TaqMan reaction, 5 µl cDNA was mixed with 1 µl water, 12.5 µl TaqMan Universal PCR Master Mix 2× (Applied Biosystem, Courtaboeuf, France), 2 µl sense primer (2 µM), 2 µl antisense primer (2 µM), and 2.5 µl TaqMan probe (2 µM). PCR parameters were 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Parallel assays using the same cDNA pools were carried out using primers and probe to the housekeeping gene porphobilinogen deaminase (PBGD, 17). Quantitative RT-PCR was performed using an ABI Prism 7700 sequence detector system (Applied Biosystem) and analyzed using relative expression to PBGD, as described previously (32). Briefly, the level of expression in the hyperplasia was normalized by dividing copies per nanogram total RNA of 5-HT₄ receptor gene by copies per nanogram total RNA of the housekeeping gene PBGD, and expressed as a percentage.

Characterization of 5-HT₄ receptor isoforms and vasopressin receptors by RT-PCR

Total RNA was extracted and reverse-transcribed as described previously. Amplification of the cDNAs encoding the different 5-HT₄ receptor C-terminal splice variants was performed by PCR using primer Fw1, which hybridizes to all 5-HT₄ receptor messengers, and splice variant-specific reverse primers (33). The 5-HT_{4(h)} variant was amplified using the forward primer Fwh, which is specific for cDNAs containing the 5-HT_{4(h)} exon, and the reverse primer Revh which hybridizes to all 5-HT₄ receptor messengers. Amplification of the cDNAs encoding the vasopressin V₁ receptor was carried out using gene-specific primers (34). Vasopressin V₂ and V₃ receptors cDNAs were amplified using the following two sets of primers: for the V₂ receptor, 5'-GTGGCCAAGACTGTGAGGAT-3' and 5'-CATAGATC-CAGGGGTTGGTG-3', corresponding to bases 1031–1050 and 1192–1211 respectively, of the human V₂ receptor cDNA (Accession no. NM000054) and for the V₃ receptor, 5'-CAGCAGCATCAACAC-CATCT-3' and 5'-CCATGTAGATCCAGGGGTTG-3', corresponding to bases 921–940 and 1122–1141 respectively, of the human V₃ receptor sequence (Accession no. AF101726). All PCR-based procedures were performed in a final volume of 50 µl containing 10% of RT mixture, 3 U DNA Taq Polymerase (Life Technologies), DNA Polymerase buffer (Life Technologies), 1.5 mM MgCl₂, 0.4 mM dNTP, and 20 pmol of each primer. The PCRs were performed for 40 cycles (94 °C, 40 s; 50 °C, 60 s; and 72 °C, 90 s). The PCR products were analyzed in 1.5% agarose gels, blotted on a nylon membrane, and hybridized with the [³²P]ATP-labeled 5-HT₄ and V₁ receptor gene-specific probes (17, 34). For the detection of V₂ and V₃ receptors, PCR products were hybridized with the following [³²P]ATP-labeled gene-specific oligonucleotides: for the V₂ receptor, 5'-GTGCTACTCATGTTGCTGGCC-3', corresponding to bases 1157–1177 of the human V₂ receptor cDNA and for the V₃ receptor, 5'-GATTCAC-CAATGTGGCTTTC-3', corresponding to bases 1063–1083 of the human V₃ receptor cDNA. In addition, PCR products were subcloned into pGEM-T (Promega) and sequenced, using the Thermosequenase kit (Amersham) on a Li-Cor 4200L DNA sequencer (ScienceTec, Les Ulis, France) using fluorescent T7 and T3 primers (MWG-Biotech, Courtaboeuf, France).

CT technique and adrenal volumetry

Two or three millimeter-thick-contiguous slices were obtained through the adrenal glands with a multislice helical CT (Sensation 16, Siemens, Erlangen, Germany) without enhancement. On each image, adrenal glands

were separately delineated with a handheld cursor. Adrenal volume was calculated automatically by the workstation (Horizon Medical Imaging, McKesson, San Francisco, USA) taking into account the delineated adrenal surfaces and the slice thickness. The normal adrenal volume was between 4 and 5 ml (35).

Statistical analysis

The nonparametric Spearman correlation test was used to study correlation between the volume of the adrenal glands and the amplitude of the abnormal cortisol responses to clinical tests (Statview 5 program, SAS Institute, Inc., Cary, NC, USA).

Results

Hormonal results

Patients III2 and III1 The two sisters were evaluated twice with an interval of 3 years. The main results are summarized in Table 1.

Patient III2. In the first evaluation, patient III2 had clinical and biochemical features of mild Cushing's syndrome (Table 1).

Three years later, there was no change in the clinical and biochemical features of Cushing's syndrome (Table 1). The patient remained untreated and underwent continuation of the follow-up.

Patient III3. During the first evaluation, patient III3 had clinical and biochemical features of mild Cushing's syndrome. The patient remained untreated.

Three years later, there was no change in the clinical features, but there was a worsening of the biochemical signs of hypercorticism (Table 1) and an increase in the size of the left adrenal (Fig. 2). The patient underwent left adrenalectomy by laparoscopy. Histopathology revealed bright yellow nodules in all the adrenal cortex which confirmed the diagnosis of macronodular hyperplasia. The internodular cortex was hyperplastic. Postoperatively, plasma cortisol was 165 nmol/l (6 µg/dl) at 0800 h and 386 nmol/l (14 µg/dl) after i.m. injection of 250 µg tetracosactide (Synacthene), which confirmed adrenal insufficiency. Treatment was started with hydrocortisone 20 mg/day and followed by a gradual decrease in the daily dose over 6 months. One month after the cessation of hydrocortisone therapy, all tests performed to study adrenal function were normal except that basal plasma ACTH at 0800 h remained low.

Patients III1 and II2 Patients III1 and II2 had biochemical features of subclinical Cushing's syndrome (Table 1).

Table 1 Evaluation of adrenal function in patients with familial adrenocorticotropin-independent macronodular adrenocortical hyperplasia.

Patient	Urinary free cortisol (nmol/day)	Midnight plasma cortisol (nmol/l)	Plasma cortisol after 1 mg dexamethasone (nmol/l)	Plasma cortisol after 2 mg dexamethasone (nmol/l)	Urinary free cortisol after 2 mg dexamethasone (nmol/day)	Basal ACTH at 0800 h (pmol/l)	CT scan and adrenal volume (ml)
II2	ND	168	77	ND	ND	5	Adrenal enlargement 10.5
III1	ND	102	72	ND	ND	3	Adrenal enlargement 9.8
III2 First evaluation	47	141	72	118	<19	1.8	Macronodular bilateral hyperplasia 27.6
III2 Second evaluation 3 years after the first one	95	201	127	ND	ND	1.5	Macronodular bilateral hyperplasia 35.8
III3 First evaluation	66	143.5	104.8	135	28.9	1.1	Macronodular bilateral hyperplasia 29.6
III3 Second evaluation 3 years after the first one	137	298	165	ND	ND	0.9	Macronodular bilateral hyperplasia 41.1
III3 Third evaluation (6 months after surgery)	27	110	28	ND	ND	2.8	6.1
IV3	ND	24.8	13.8	ND	ND	4.6	Normal adrenal CT scan 7
IV4	ND	121.4	27.6	ND	ND	3.3	Normal adrenal CT scan 5
IV5	ND	30.3	22.1	ND	ND	10.8	Normal adrenal CT scan 6
Normal reference	<208	<138	<50	<90	<28	>3.3	4–5

ND, not done.

Patients IV3, IV4, and IV5 All these patients had no biochemical features of Cushing's syndrome (Table 1).

CT scans

The main results are shown in Fig. 2 and Table 1.

In patient III2, CT scan revealed bilateral enlargement of the adrenal glands with multiple nodules. Three years later, the mean volume of the adrenal glands had increased by 30%.

On the first evaluation, CT scan of patient III3 showed a macronodular bilateral hyperplasia that was more pronounced in the left adrenal. Three years later, the mean volume of the adrenals had increased by 39%.

In patients III1 and II2, adrenal CT scan showed two thick adrenals without any nodule.

CT scans showed normal adrenal glands in patients IV3, IV4, and IV5.

Correlation between adrenal gland volume and hormonal results There was a significant positive correlation between the volume of the adrenals and

plasma cortisol level after 1 mg overnight dexamethasone test ($P=0.008$, Fig. 3), and between the volume of the adrenals and midnight plasma cortisol ($P<0.02$; data not shown). A significant negative correlation was also observed between the volume of the adrenals and plasma ACTH concentration at 0800 h ($P=0.02$; data not shown).

In vivo screening tests for aberrant adrenal receptors expression

In vivo screening for aberrant adrenal receptors was performed in patients III1, III2, III3, and IV4 (Tables 2 and 3). Stimulation tests with glypressin, cisapride or metoclopramide, and glucagon were performed twice with an interval of 3 years in patients III2 and III3 (Tables 2 and 3).

In vivo pharmacological tests showed a significant increase in plasma cortisol after terlipressin and cisapride in patients III2 and III3 (Tables 2 and 3). Three years later, a significant response was found in

Patient II1

Patient III1

Patient III2

First evaluation

Second evaluation

Patient III3

First evaluation

Second evaluation

Patient IV4

Figure 2 CT scan of patients II2, III1, III2, III3, IV3, IV4, and IV5.

Figure 3 Correlation between adrenal volume and plasma cortisol after 1 mg overnight dexamethasone test in patients with familial AIMAH. To convert plasma cortisol $\mu\text{g/dL}$ to system international units (nmol/l), multiply by 27.59. Data points are: 1, patient IV4; 2, third evaluation of patient III3 (6 months after surgery); 3, patient IV3; 4, patient IV5; 5, patient III1; 6, patient II2; 7, first evaluation of patient III2; 8, first evaluation of patient III3; 9, second evaluation of patient III2, 10, second evaluation of patient III3.

both patients, after terlipressin and metoclopramide (Tables 2 and 3).

In vivo screening for adrenal aberrant receptors was negative in patients III1 and IV4 (Table 2).

***In vitro* detection of adrenal aberrant receptors in the adrenal tissue obtained from patient III3**

Expression profile of 5-HT₄ receptors in the hyperplasia tissue The relative amount of 5-HT₄ receptor mRNA in hyperplasia tissue was determined by real-time RT-PCR. When expressed as arbitrary units normalized to PBGD, 5-HT₄ receptor level was six times higher than the mean level previously observed in normal adrenal glands, i.e., 12.2 vs. 2.13% in normal adrenals (17). RT-PCR amplification was applied to characterize 5-HT₄ receptor isoforms and vasopressin receptors in the hyperplastic tissue. The tissue was

found to express isoforms (a), (b), (c), (i), and (n) of the 5-HT₄ receptor whereas isoform (i) was not detected in a normal adrenal cortex (Fig. 4). Individual bands obtained from hyperplasia reverse-transcribed RNAs were excised, ligated into pGEM-T, and sequenced. All sequences corresponded to the published sequence of the 5-HT₄ receptor cDNA (data not shown).

Expression profile of vasopressin receptors in the hyperplasia tissue V₁ and V₂ receptor PCR products were detected in the hyperplasia extract. In contrast, the V₃ receptor mRNA was not expressed in the tissue (Fig. 4). In agreement with previous observation (8), the normal adrenal cortex was found to exclusively express the V1 receptor. The two bands obtained from hyperplasia reverse-transcribed RNAs were excised, ligated into pGEM-T, and sequenced. The sequences corresponded to the published sequences of the V₁ and V₂ receptor cDNAs respectively (data not shown).

Discussion

Familial ACTH-independent Cushing's syndrome with bilateral macronodular hyperplasia has only recently been described in a limited number of families (20–24). Moreover, only two of these studies had evaluated *in vivo* and *in vitro* expressions of aberrant adrenal receptors in these families (20, 24). We describe a new case of familial AIMAH investigated for the presence of aberrant adrenal receptors. The two sisters had a mild form of Cushing's syndrome with macronodular bilateral hyperplasia and *in vivo* and *in vitro* expressions of 5-HT₄ and vasopressin adrenal aberrant receptors. Their father and brother had moderate adrenal enlargement with features of subclinical Cushing's syndrome. *In vivo* screening tests failed to detect adrenal aberrant receptors in the brother. The other members of the family who were evaluated, exhibited no features of AIMAH.

Table 2 *In vivo* screening for adrenal aberrant receptors for patient III1, III2, III3, and IV4.

Patient	Posture test	Meal test	LHRH	Terlipressin	Desmopressin	Metoclopramide	Cisapride	Glucagon	TRH	ACTH 1–24
III1	5	–10	–1	3	ND	28	ND	8	–3	746
III2 First evaluation	31	–4	8	106	–25	ND	127	25	–26	382
III2 Second evaluation	ND	ND	ND	165	ND	170	ND	0	ND	ND
III3 First evaluation	25	–6	3	192	16	ND	220	–5	18	654
III2 Second evaluation	ND	ND	ND	158	ND	201	ND	+4	ND	ND
IV4	–4	–18	–43	–42	ND	–14	ND	29	0	330

Data are the percentage of increase above the basal value. ND, not done.

Table 3 Results of cortisol and adrenocorticotropin during terlipressin, metoclopramide, and cisapride tests in patients III2 and III3.

	T0	T30	T60	T90	T120
<i>Patient III2</i>					
Terlipressine test first evaluation					
Cortisol (nmol/l)	342	692	706	543	508
ACTH (pmol/l)	1.3	1.3	1.1	1.1	0.9
Terlipressine test second evaluation					
Cortisol (nmol/l)	135	359	342	223	270
ACTH (pmol/l)	0.9	0.9	1.1	0.9	1.1
Cisapride test first evaluation					
Cortisol (nmol/l)	204	290	411	464	408
ACTH (pmol/l)	0.9	0.9	1.1	1.1	0.9
Metoclopramide test second evaluation					
Cortisol (nmol/l)	229	210	428	618	585
ACTH (pmol/l)	1.3	0.9	1.1	1.1	0.9
<i>Patient III3</i>					
Terlipressine test first evaluation					
Cortisol (nmol/l)	270	789	590	499	430
ACTH (pmol/l)	0.9	0.7	0.7	0.9	0.9
Terlipressine test second evaluation					
Cortisol (nmol/l)	179	464	439	383	353
ACTH (pmol/l)	1.1	0.9	0.7	0.7	0.9
Cisapride test first evaluation					
Cortisol (nmol/l)	315	447	784	894	1007
ACTH (pmol/l)	1.3	1.1	1.1	0.7	0.7
Metoclopramide test second evaluation					
Cortisol (nmol/l)	215	212	458	648	648
ACTH (pmol/l)	0.9	1.1	0.7	0.7	1.1

In sporadic AIMAH, Cushing's syndrome occurs more frequently in the fifth decade and remains moderate despite the large size of adrenal nodules (review in 36). Unlike sporadic AIMAH, in previous reports on familial AIMAH, Cushing's syndrome seems to occur at various ages (30–70 years) and Cushingoid features can be moderate or more pronounced (20–24). In our familial AIMAH study, the clinical features are similar to those of most of AIMAH sporadic forms. Patients III2 and III3 were in their fifth decade and had only mild clinical expression of Cushing's syndrome. The urinary free cortisol level was still in the normal range indicating that Cushing's syndrome remained moderate (25). The moderate expression of Cushing's syndrome despite the large adrenal size could be explained by enzymatic defects in AIMAH (37, 38). Indeed, the main histopathologic components of AIMAH are clear and compact cells which show weaker activity for steroidogenic enzymes (39). Furthermore, we show that, in this family with AIMAH, there is a significant positive correlation between the volume of the adrenal glands and the abnormalities of cortisol secretion. To our knowledge, it is the first report of such correlation within family with AIMAH. This could explain that the clinical manifestations of Cushing's syndrome occur at a later age in AIMAH when compared with the other causes of Cushing's syndrome. The diagnosis of Cushing's syndrome with AIMAH could be made only when adrenal volume reaches a minimal threshold.

In the previous two reports on familial AIMAH with the evaluation of aberrant receptors, the expressions of LH/HCG and/or vasopressin adrenal receptors were observed. We report for the first time that serotonergic receptors can be expressed in familial AIMAH. The expression of adrenal aberrant receptors in familial AIMAH seems also similar to that observed in sporadic forms (1, 4, 17, 40, 41). Indeed, most of sporadic AIMAH and unilateral adrenal adenomas express adrenal aberrant receptors (42–44). Usually, the more frequently expressed receptors in AIMAH are V1 receptor and 5-HT4 serotonergic receptors (1, 6, 11). Moreover, we report that, as in sporadic AIMAH (11), several isoforms of each aberrant adrenal receptor can be expressed in familial AIMAH. The two vasopressin receptors subtypes expressed in the tissue removed from patient III3 could have a role in the control of cortisol secretion and may explain the abnormal *in vivo* cortisol response to terlipressin. The cortisol production due to overexpression or increased activity of eutopic V1 receptor could be potentiated by ectopic expression of V2 receptor. Moreover, several isoforms of 5-HT4 receptor have been found in patient III3 adrenal tissue as in normal adrenals (17). The isoforms detected in this hyperplasia were similar to those expressed in normal adrenals except for the isoform (i). Indeed, isoform (i) was present in the patient's tissue but was expressed very weakly in only few normal adrenals (33). To our knowledge, the detection of isoform (i) had never been reported in sporadic or familial AIMAH; whether such isoform

Figure 4 RT-PCR analysis of 5-HT4 receptor isoforms a–i and n, and vasopressin receptors V1, V2, and V3 in hyperplasia tissue from patient III3 and a normal adrenal cortex. RT-PCR amplification was used pairs of primers specific for the different splice variants.

expression plays a role in the pathogenesis of familial or sporadic AIMAH remains to be elucidated.

We also confirm that, in some cases, *in vivo* screening cannot detect all the receptors expressed by the hyperplastic adrenals (6). In patient III3, *in vivo* desmopressin test was negative despite *in vitro* ectopic expression of V2 vasopressin receptors. Clinical tests could detect aberrant adrenal receptors only when the expression level of aberrant receptors reaches a significant threshold. We can speculate that this threshold could be related to the volume of the adrenal glands.

As in sporadic cases (45), unilateral adrenalectomy can be effective to control Cushing's syndrome. Treatment of sporadic AIMAH is classically bilateral adrenalectomy but a specific medical treatment can be successfully proposed to those patients who express LH/hCG, β -adrenergic, and GIP adrenal aberrant receptors (review in 1). However, specific 5-HT4 and/or V1 receptor antagonists are not currently available. In the present study, unilateral adrenalectomy with the removal of the larger adrenal appeared as a valuable therapeutic choice for AIMAH patients with Cushing's syndrome (24, 45). Transient adrenal insufficiency occurred probably because of relative inefficient steroidogenesis in patients with AIMAH. The contralateral gland may be transiently unable to maintain appropriate cortisol levels, despite showing macronodular hyperplasia. In addition, ACTH levels remained suppressed right after surgery, so that there was no ACTH-mediated increase in cortisol secretion by the hyperplastic adrenal gland.

Several differences between sporadic and familial AIMAH histopathology can be underlined. In sporadic AIMAH, histopathology can show either atrophic or hyperplastic internodular cortex. In contrast, atrophic internodular adrenal cortex has never been reported in familial AIMAH. In accordance with previous reports on familial AIMAH and especially that on familial AIMAH with aberrant expression of vasopressin and

LH/hCG adrenal receptors (21, 24), we establish that the internodular adrenal cortex is hyperplastic in familial AIMAH. However, further studies are required to confirm that the presence of internodular hyperplasia may suggest familial AIMAH.

The pathogeny of sporadic and familial AIMAH remains unclear. Familial AIMAH provides evidence that genetic transmission of the disease can occur. In our observation, as in the previous two reports on familial AIMAH, which had studied two generations of the same family (22, 24), AIMAH seems to be an autosomal dominantly inherited disease. Nonetheless, phenotypic expression seems to be more pronounced in female than in male patients. In our study, only women in the fifth decade of life had cortisol hypersecretion sufficient to lead to mild clinical and biochemical Cushing's syndrome. The two men had only moderate hormonal abnormalities with subclinical Cushing's syndrome. Similarly, the radiological presentation of the disease was different in affected women versus men. The two sisters had macronodular bilateral hyperplasia, while the two men had only a moderate adrenal enlargement. Although the genetic transmission of the disease seems to be autosomal dominant, the gender seems to influence the phenotypic expression regarding both cortisol secretion and adrenal cells proliferation. Consistent with this hypothesis, another group described a family with AIMAH in whom phenotypic expression of the disease was more pronounced in female than in male patients (22). Moreover, *in vivo* and *in vitro* studies showed that, in an adrenal micronodular dysplasia, the secretion of cortisol could be stimulated by estrogens (46). Therefore, the phenotypic expression in AIMAH could partly depend on sex steroids. Further studies are necessary to elucidate the possible role of sex steroids in the pathogenesis of familial or sporadic AIMAH.

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