Clinical and Subclinical ACTH-Independent Macronodular Adrenal Hyperplasia and Aberrant Hormone Receptors

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Key Words

Adrenocortical hyperplasia · ACTH-independent macronodular adrenal hyperplasia · Cushing's syndrome, subclinical · Aberrant receptors

Abstract

ACTH-independent macronodular adrenal hyperplasia (AIMAH) is a very rare cause of endogenous Cushing's syndrome (CS). In this review, the clinical characteristics, the pathophysiology, and the management of AIMAH are described. AIMAH typically presents with overt CS, but subclinical oversecretion of cortisol has been increasingly described. The diagnosis is suspected by adrenal nodular enlargement on conventional imaging following the demonstration of ACTH-independent hypercortisolism. Final diagnosis is established by histological examination of the adrenal tissue. Bilateral adrenalectomy is the treatment of choice but unilateral adrenalectomy has been proposed in selected cases. In patients with subclinical CS, the decision to treat should be individualized. The pathophysiology of this condition has begun to be elucidated in recent years. Diverse aberrant membrane-bound receptors expressed in a non-mutated form in the adrenal gland have been found to be implicated in the regulation of steroidogenesis in

AIMAH. When systematically screened, most patients with AIMAH and CS or subclinical CS exhibit an in vivo aberrant cortisol response to one or various ligands suggesting the presence of aberrant adrenal receptors. A protocol designed to screen patients for the presence of these aberrant receptors should be undertaken in all patients with AIMAH. The identification of these receptors provides the potential for novel pharmacological therapies by suppressing the endogenous ligands or blocking the receptor with specific antagonists.

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Introduction

Endogenous Cushing's syndrome (CS) is due to primary adrenal hypersecretion in approximately 15–20% of cases [1]. Unilateral adrenal pathology, in the form of adrenocortical adenomas or carcinomas, represents the large majority of adrenal CS. Bilateral adrenal pathology causing CS represents only 10–15% of all cases and includes: (1) primary pigmented nodular adrenocortical disease (PPNAD) commonly associated with Carney complex; (2) ACTH-independent macronodular adrenal hyperplasia (AIMAH) [1], and rarely (3) bilateral adenomas or carcinomas. AIMAH is thus a very rare cause of CS representing less than 1% of its endogenous etiologies. In 1994, Lieberman et al. [2] reviewed 24 published cases. Since then, a much greater number of cases have been reported and the characteristics of this distinct entity

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have increasingly been delineated. A variety of terms have been used in the literature to label this condition including: 'massive macronodular adrenocortical disease'; 'autonomous macronodular adrenal hyperplasia'; 'ACTH-independent massive bilateral adrenal disease'; 'giant or huge macronodular adrenal hyperplasia', and 'macronodular adrenal dysplasia' [3, 4].

In this review, we will present the clinical characteristics of AIMAH, the recent data on the pathophysiology of this condition, most notably the role of aberrant adrenal receptors, and finally its treatment.

Clinical Features

AIMAH was first described in 1964 by Kirshner et al. [5] who reported the case of a 40-year-old woman with long-standing CS. They demonstrated that her hypercortisolism was ACTH-independent and that the resected adrenal glands contained multiple nodules with a combined weight of 94 g. Since then, a number of cases have been described in the literature and the clinical characteristics of this legitimate cause of CS have been more precisely characterized.

Epidemiology

AIMAH appears to have a bimodal age distribution, with a subset of patients presenting in the first year of life [6], usually associated the McCune-Albright syndrome. Most patients present in the 5th and 6th decades [2–4, 7], representing a much later age at onset compared with unilateral cortisol-producing adenomas or pituitary corticotroph adenomas. Furthermore, this later age at onset differs from that of PPNAD, which is around 18 years of age [8]. In contrast to the predominant female distribution in most causes of endogenous CS, AIMAH appears to be equally distributed between genders [2, 3, 7]. One report suggested an increased frequency in males [9] and another an increased occurrence of gastric inhibitory polypeptide (GIP)-dependent AIMAH in women [10]. In the majority of cases, AIMAH appears to be sporadic. However, there have been reports of familial clustering with the suggestion of an autosomal dominant pattern of transmission [2, 11–15].

Clinical Presentation

AIMAH cases are discovered either because of an incidental radiological finding or following the investigation of an adrenal hypersecretion syndrome. The most common clinical presentation is CS. At the time of diag-

nosis, subtle signs and symptoms consistent with CS have typically been present for a number of years. In one series, the diagnosis was delayed by a mean of 7.8 years [4]. As in any cause of adrenal cortisol hypersecretion, plasma ACTH is suppressed and the high-dose dexamethasone suppression test fails to suppress cortisol production. In recent years, the occurrence of subclinical CS defined as the absence of clinical signs of CS, slightly elevated midnight plasma or salivary cortisol, subnormal suppression following the 1-mg overnight dexamethasone suppression test (>50 nmol/l), a partially suppressed ACTH, and a normal 24-hour urinary cortisol has been shown to occur with AIMAH. Bourdeau et al. [16] described 4 patients referred for incidentally encountered bilateral adrenal macronodular glands in whom the presence of subclinical CS was demonstrated. The natural history of AIMAH causing subclinical CS is largely unknown. Bourdeau et al. [16] reported a stable clinical course and maintenance of normal urinary free cortisol levels in 2 of the 4 patients described in whom 12-month follow-up was available. Ohashi et al. [17] reported a 7-year follow-up during which a patient with subclinical CS developed elevated 24-hour urinary free cortisol, a significant increase in the total volume of the adrenal glands, and overt CS.

Concurrent secretion of cortisol and mineralocorticoids [18–20] or of cortisol and estrone [21] have been described in patients with AIMAH. Goodarzi et al. [22] reported the case of a 59-year-old woman with pure androgen overproduction associated with macronodular adrenal hyperplasia.

Radiologic Features

The diagnosis of AIMAH and CS is suspected by typical imaging studies following the biochemical demonstration of ACTH-independent CS. Doppman et al. [23] reviewed the imaging features in 12 patients with surgically proven AIMAH. On CT scan, bilateral adrenal masses measuring up to 5 cm of soft tissue density usually distort and completely obscure the normal adrenal glands (fig. 1a). In some cases, the adrenal glands appear diffusely enlarged but lack distinct nodules. On MRI, T1-weighted images are hypo-intense relative to the liver and iso-intense relative to muscle. T2-weighted images tend to be hyper-intense relative to the liver [23, 24]. In contrast, the nodules of pituitary-dependent macronodular hyperplasia due to chronic ACTH stimulation appear iso-intense relative to the liver on T2-weighted MR images [23]. The asymmetric appearance of adrenal macronodules in AIMAH has been described [2, 3, 25] and may lead to the erroneous diagnosis of unilateral patholo-

Fig. 1. a CT scan of a patient with AIMAH demonstrating bilateral adrenal nodular enlargement. **b** Gross macroscopy of a macronodular adrenal gland from a patient with AIMAH.

gy. Iodine-131-6 β -iodomethyl-19-norcholesterol (NP-59) scintigraphy typically shows bilateral uptake [26].

Pathologic Features

The combined adrenal weight is usually greater than 60 g and can reach more than 200 g/gland. The mean combined weight in one series was 132 g [23]. In comparison, the combined adrenal weight in patients with ACTH-dependent Cushing's disease has been reported to be 22.9 g on average in a series of 30 patients [27]. On cut sections, the nodules are yellow due to their high lipid content (fig. 1b) [9]. Final diagnosis is established by histological examination of the adrenal tissue. The nodules are composed of two cell types: Those with a clear cytoplasm (lipid-rich) that form cordon nest-like structures, and those with a compact cytoplasm (lipid-poor) that form nests or island-like structures [9, 28]. A characteristic finding is inter-nodular hyperplasia, although atrophy has been described in several cases [2, 4, 8, 9, 29, 30]. AIMAH is a benign process that has never been shown to acquire a malignant potential or to metastasize.

Differential Diagnosis

AIMAH is distinguished from the other type of bilateral nodular adrenal hyperplasia PPNAD by: (1) a later age at onset; (2) the presence of massive macronodular

adrenal hyperplasia as assessed radiologically compared with normal or slightly enlarged micronodular glands seen with PPNAD; (3) the absence of deeply pigmented nodules that appear black or brown on cut section, and (4) the inter-nodular cortical hypertrophy that contrasts with the prominent atrophy observed between nodules in PPNAD.

Pathophysiology

Steroidogenesis in AIMAH

Steroid hormone synthesis has been shown to be an inefficient process in AIMAH. Thus, the cortisol hypersecretion is the result of a significantly increased number of adrenocortical cells rather than a more efficient steroidogenesis. This compromised hormone synthesis is the consequence of altered steroidogenic enzymatic pathways. Immunohistochemical studies have revealed a differential expression of 3 β -HSD2 and CYP-17 in AIMAH that is not observed in other forms of adrenocortical pathologies: The former enzyme is exclusively expressed in large clear cells, whereas the latter is mainly expressed in small compact cells [31–33]. Immunoreactivity studies demonstrate the expression of other steroidogenic enzymes such as CYP11A1, CYP21A2, CYP11B2 to be present in both cell types, but at a decreased level, further

contributing to the inefficiency of steroid synthesis [28, 32, 34, 35]. Furthermore, elevated 17-hydroxyprogesterone levels have been shown to occur following stimulation with ACTH providing in vivo evidence of altered adrenocortical enzymatic activity [36]. This inefficient steroidogenesis may explain the discrepancy between the sometimes subtle changes in cortisol hypersecretion such as seen in subclinical CS and the massive adrenal enlargement.

Molecular Mechanisms of AIMAH

'Autonomy' Theory

In recent years, the pathogenic mechanisms that lead to adrenal tumorigenesis and hypercortisolism have begun to be elucidated. An early theory for the pathogenesis of AIMAH hypothesized that chronic stimulation of the adrenal cortex by ACTH in long-standing Cushing's disease or ectopic ACTH secretion would lead to a hyperplastic adrenal cortex that progressively acquired adrenal autonomy [27, 37, 38]. Smals et al. [27] compared the adrenal glands of patients with AIMAH to those in patients with long-standing Cushing's disease and concluded that prolonged adrenal stimulation by ACTH resulted in adrenal bilateral nodular formation and varying degrees of adrenal autonomy. A few cases have been described in which autonomy of the adrenal gland was the result of chronic ACTH stimulation and eventually resulted in ACTH suppression [39–42]. However, the rarity of Nelson's syndrome following bilateral adrenalectomy in patients with AIMAH strongly argues against the hypothesized transition from ACTH-dependency to adrenal autonomy. In a series of 9 patients with AIMAH followed for 8.5 years after undergoing bilateral adrenalectomy, none of the patients developed Nelson's syndrome [4]. Further evidence for a primary adrenal alteration was provided by Cheitlin et al. [43] who used in vitro cell cultures from a patient with AIMAH and demonstrated a high rate of growth and maintained cortisol production despite the absence of ACTH in the culture medium.

MC2R Mutations

The possibility of a mutated ACTH receptor (*MC2R*) leading to constitutive receptor activation has been examined. *MC2R* mutations have not been found to be a common cause of adrenal hyperplasia or tumor formation [44–46]. Swords et al. [47] reported a patient with AIMAH in whom a *MC2R* mutation (F278C) in the C-terminal of the receptor led to impaired desensitization and internalization of the receptor and consequently elevated basal cAMP. The same authors recently described

a patient with clinical hypersensitivity to ACTH resulting from two mutations in the same allele of *MC2R*. The presence of either mutation alone (C21R or S247G) produced an inactive receptor but the presence of both mutations resulted in constitutive receptor activity [48].

Gsp Mutations

Alterations in the signaling pathways downstream of the ACTH receptor have also been implicated in the pathogenesis of adrenal hyperplasia and cortisol hypersecretion. In the McCune-Albright syndrome (MAS), activating mutations of the Gs α subunit can occur in a mosaic pattern in the adrenal gland during early embryogenesis resulting in the constitutive activation of the cAMP pathway. The association of MAS with bilateral nodular adrenal hyperplasia and CS has been described in a number of reports in the literature [6, 49–54]. Fragoso et al. [46] identified two different *gsp* mutations at codon Arg²⁰¹ in 3 of 5 patients with CS due to histologically confirmed AIMAH and who exhibited no signs of MAS. These cases may represent variants of MAS or may be the result of late somatic mutations. However, another report failed to identify any *gsp* mutation using direct sequencing in 13 cases of AIMAH [55].

Association with MEN1 and Familial Adenomatous Polyposis

Bilateral adrenal nodules have also been associated with MEN1, which is caused by a mutation of the tumor suppressor gene *MENIN*. Bilateral adrenal enlargement was found in 21% of a series of 33 MEN1 patients [56]. In a large kindred of MEN1, the prevalence of bilateral macronodular adrenal hyperplasia was 6%. To our knowledge, a sporadic somatic mutation of the *MENIN* gene in adrenocortical tissue in AIMAH has never been described. Bilateral adrenal nodules have also been reported in patients with familial adenomatous polyposis, but a somatic point mutation of the *APC* gene in the hyperplastic adrenal tissue has never been described [57, 58].

Gene Microarray Analysis

In a gene expression profile study of tissues from 8 AIMAH patients using large-scale cDNA microarray analysis, 82 genes were found to be upregulated, mainly genes involved in transcription, the cell cycle and adhesion; 31 genes, including those responsible for adrenal steroidogenesis, were downregulated [35]. Further work to elucidate the mechanisms by which these genes are up- or downregulated will shed some light on the molecular pathways involved in adrenal tumorigenesis.

Table 1. Aberrant adrenal receptors in AIMAH

Aberrant receptor	In vivo screening protocol	Medical treatment options ¹
GIP receptor	Mixed meal	Octreotide
Vasopressin receptor (V1, V2, V3)	Upright posture Administration of arginine-vasopressin	V1a receptor antagonist
β -Adrenergic receptor	Upright posture	β -Adrenergic antagonists
LH/hCG receptor	GnRH administration	Long-acting GnRH agonist
5-HT ₄ receptor	Administration of 5-HT ₄ receptor agonists (cisapride or metoclopramide)	5-HT ₄ receptor antagonist
Angiotensin receptor	Upright posture	AT-1 receptor antagonist

¹ Medical treatment options include proven effective treatments as well as theoretical pharmacologic therapies.

Aberrant Hormone Adrenal Receptors in Adrenal CS (table 1)

The mechanism by which cortisol production is stimulated in AIMAH in the face of suppressed ACTH levels was previously unknown and was considered to be an 'autonomous' process. Recent work by several groups has demonstrated that cortisol secretion in most AIMAH and some adrenal adenomas is regulated by hormones other than ACTH via the ectopic expression or the overactive eutopic expression of several membrane-bound hormone receptors. Most of these receptors belong to the superfamily of G protein-coupled receptors that have become aberrantly coupled to steroidogenesis. This concept was first proposed by Schorr and Ney [59] who demonstrated in vitro that corticosterone production in adrenocortical carcinoma cells was stimulated by hormones other than ACTH, such as catecholamines and TSH.

GIP-Responsive AIMAH

Food-dependent cortisol production was first identified in a patient with a cortisol-producing unilateral adenoma [60]. Two well-characterized in vivo examples of GIP-dependent hypercortisolism were described simultaneously by 2 independent groups in 1992 [61, 62]. It was observed in both patients with AIMAH and CS that cortisol production was stimulated by the postprandial physiological increase in plasma levels of GIP. Plasma cortisol levels were correlated with plasma GIP levels during various test meals. The presence of GIP receptors was further supported by adrenal imaging following the

injection of [¹²³I]-GIP [61]. GIP-dependent CS has now been described in over 17 patients with AIMAH [10, 25, 61–67] and in 7 patients with unilateral adenomas [10, 60, 68–71]. GIP-dependent oversecretion of both androgen and cortisol has been described in a patient with an adrenal adenoma presenting with hyperandrogenic symptoms [71]. The GIP receptor is ectopically expressed in the adrenal gland in these patients as it is normally not expressed in the normal fetal or adult adrenal cortex. It is expressed at the cell surface in a non-mutated form as assessed by sequence analysis of the full-length cDNA [68, 71, 72]. The expression of the GIP receptor can be detected in the early phases of adrenal hyperplasia [25]. In vitro, GIP increases cAMP production as well as DNA synthesis in GIP-dependent cortisol-secreting adenoma cells in primary culture, suggesting that GIP induces both hormonogenesis and cell proliferation [68]. The demonstration that bovine adrenal cells transfected with the GIP receptor and injected under the renal capsule in mice lead to the development of hyperplastic adrenals and hypercortisolism further supports the initiation role of the ectopic receptor in the pathophysiology of AIMAH [73].

Vasopressin-Responsive CS

Aberrant stimulation of cortisol secretion by vasopressin has been reported in adrenal CS independent of ACTH stimulation. A number of patients with both unilateral adenomas and AIMAH have been described in whom exogenous vasopressin or physiological stimuli of vasopressin secretion such as upright posture stimulate

the secretion of cortisol [15, 74–77]. In the majority of these cases, it was demonstrated that the action of vasopressin was mediated via non-mutated V1-vasopressin receptors that are expressed at similar or higher levels compared with controls [10, 15, 57, 74–83]. An exaggerated calcium flux and cortisol secretion were observed in vitro in adrenal cells incubated with vasopressin and these effects were inhibited by a V1-vasopressin receptor antagonist [79, 82, 84]. Given that the V1 receptor is normally expressed in the adrenal cortex and that its activation leads to a modest in vitro increase in steroidogenesis, the observed exaggerated steroidogenic response represents an aberrant response of a eutopic receptor. This may be the result of receptor overexpression and/or of a more efficient intracellular coupling to the steroidogenic pathways. The ectopic expression of V2- and V3-vasopressin receptors has been documented in vitro in adrenal tissue from patients with AIMAH; however, the in vivo demonstration of the effect of V2-vasopressin receptor in these cases is lacking [15, 76].

Catecholamine-Responsive AIMAH

In human adrenal glands, catecholamines have a direct modulatory effect on aldosterone, but not cortisol, secretion. The ectopic expression of β -adrenergic receptors was first reported in vitro in tissue from adrenal tumors associated with CS [10]. In vivo, aberrant responses to elevations in endogenous catecholamines as induced by upright posture, insulin-induced hypoglycemia and exercise were documented in 4 patients with AIMAH and CS [83, 85–87]. Furthermore, isoproterenol infusion stimulated cortisol and aldosterone secretion in these patients but failed to do so in normal subjects. These aberrant responses were reduced by pretreatment with propranolol, a β -adrenergic antagonist. Two cases have been reported in which combined aberrant responses of both β -adrenergic and V1-vasopressin receptors were demonstrated in vivo [83, 87]. High-affinity binding sites compatible with β 1- and β 2-adrenergic receptors have been identified in the adrenal tissues of such patients and have been shown to be coupled functionally to steroidogenesis [86]. Further studies are needed to better characterize the subtype of adrenergic receptors present in these patients and to determine whether they are expressed in a non-mutated form.

LH-Responsive AIMAH

The LH/hCG receptor is normally mainly expressed in the gonads [88]. In the human adrenal, this receptor has been identified by immunohistochemistry to be pres-

ent only in the zona reticularis [89]. In the fetal adrenal gland, hCG stimulates DHEAS secretion [90]. The first description of an aberrant expression of the LH/hCG receptor was that of a woman with AIMAH who had transient CS during pregnancy and persistent CS following menopause [91]. In this patient, cortisol secretion was stimulated by the exogenous administration of GnRH, hCG, or recombinant LH. The administration of the long-acting GnRH agonist leuprolide acetate led to the suppression of endogenous LH production and to the subsequent normalization of cortisol secretion. The LH/hCG receptor was present in a non-mutated form and its expression was not increased compared with normal adrenal tissue [92]. In the same patient, plasma cortisol, free testosterone, and DHEAS were increased by oral intake of cisapride and metoclopramide, two serotonin 5-HT4 receptor agonists [75]. Another 2 patients were described with combined aberrant responses to LH/hCG receptor agonists and 5-HT4 agonists [93]. Bourdeau et al. [16] described 2 patients with subclinical CS who showed responses to both hCG and 5HT-4 in vivo. There has also been a report of a patient with combined aberrant responses to both hCG and GIP [94].

Serotonin-Responsive AIMAH

In the normal adrenal gland, 5-HT4 receptor agonists are potent stimulators of aldosterone secretion but only weakly affect cortisol secretion in vitro. In vivo, they normally do not produce an increase in plasma cortisol [95]. Cartier et al. [96] reported 6 cases of CS and AIMAH with aberrant responses to cisapride and demonstrated adrenal overexpression of the 5-HT4 receptor in the adrenal glands of 4 of these patients. Another group described a patient with cisapride-responsive CS and AIMAH in whom adrenal overexpression of the 5-HT4 receptor was also found [97]. A case of hyperaldosteronism and cyclical Cushing's with aberrant responses to both aldosterone and cortisol to 5-HT4 agonists has also been reported [20]. In addition to the eutopic overexpression of 5-HT4 receptors described above, ectopic expression of 5-HT7 receptors has been demonstrated in patients with AIMAH and CS [76].

Angiotensin-Responsive AIMAH

The possibility of adrenal hypersensitivity to angiotensin II was entertained in a patient with AIMAH and CS in whom a large increase in plasma aldosterone and cortisol were noted during upright posture [98]. The short-term oral administration of candesartan, an AT-1 receptor antagonist, completely eliminated the stimulation of

Fig. 2. Investigative protocol to further characterize abnormal adrenal hormone receptors following the initial screening protocol. Reproduced with permission from Lacroix et al. [108].

these adrenal hormones. An angiotensin infusion was not performed nor was a trial of long-term pharmacotherapy with an AT-1 receptor blocker attempted. In vitro stimulation of cortisol secretion in adrenal tissue by angiotensin II from patients with AIMAH has been shown but the presence of the AT-1 receptor has never been demonstrated [99].

Other Abnormal Responses in AIMAH

Leptin synthesis is stimulated by glucocorticoids and this hormone normally inhibits cortisol secretion in the adrenal gland [100–102]. In a patient with AIMAH and CS, GIP and leptin were shown to aberrantly increase cortisol production in vitro. The GIP and leptin receptors were not measured directly in this case [64]. These responses have never been demonstrated in vivo. Two patients with AIMAH and CS have been described in whom

insulin-induced hypoglycemia stimulated cortisol production while ACTH levels remained undetectable [103, 104]. In vitro, adrenal cortisol secretion was not stimulated by insulin, catecholamines, vasopressin, or angiotensin II [104]. Other in vitro studies of adrenal material from both benign and malignant lesions have suggested the expression of other aberrant membrane receptors such as thyrotropin, FSH, and IL-1 [10, 105, 106].

A patient with AIMAH and CS was recently described in whom an increased adrenocortical expression of POMC/ACTH was described, thus suggesting a paracrine regulatory mechanism of hypercortisolism [107].

Investigative Protocol for Aberrant Receptors

An investigative protocol to screen patients with adrenal Cushing's for aberrant receptors has been developed and has been previously described in detail [108]. The

strategy consists of modulating the plasma levels of various hormones (endogenous or exogenous) or pharmacologic receptor ligands while monitoring levels of plasma ACTH, cortisol, and other steroid hormones.

All tests are performed following an overnight fast and in a supine position for at least 1 h. The initial screening is performed over the course of 3 days and involves: (1) a posture test to screen for receptors to angiotensin II, vasopressin, catecholamines, or atrial natriuretic peptides; (2) ingestion of a standard mixed meal to assess the presence of GIP or other gastrointestinal hormone receptors; (3) administration of GnRH to screen for responses to GnRH, LH, and FSH; (4) administration of TRH to screen for modulation by TRH, TSH, or prolactin; (5) administration of glucagon; (6) administration of arginine-vasopressin, and (7) administration of cisapride or metoclopramide, serotonin 5-HT₄ receptor agonists. Serial measurements of ACTH and cortisol are performed at 30- to 60-min intervals over the course of 2–3 h following the intervention. A change of 25–49% from the baseline cortisol levels in the absence of an increase in ACTH is defined as a partial response and a change of 50% or greater is considered a positive response to the specific ligand [10]. If a partial or positive cortisol response is elicited, the test is repeated to assess its reproducibility and to examine whether or not other steroids are simultaneously stimulated. Fluctuations of the ligand hormones of interest are also measured to better define the modulator of the response. If a positive response following this initial screening is clearly documented, further stimulatory tests are undertaken in order to precisely implicate a hormone or a more specific receptor type (fig. 2).

Frequency of Aberrant Receptor Responses in AIMAH

Individual case reports fail to provide information on the frequency of aberrant receptors in AIMAH. In a systematic clinical screening of 20 consecutive patients with adrenal CS, all 6 patients with AIMAH had evidence of at least one aberrant receptor type: 2 GIP receptors; 1 V1 vasopressin receptor; 1 LH/hCG receptor and 5-HT₄ receptor; 1 β -adrenergic receptor, and 1 β -adrenergic receptor and V1-vasopressin receptor [83]. In the same series, aberrant receptor responses were identified in only 3 of 13 patients with unilateral adenomas and none in one adrenal carcinoma. In a similar study by the French COMET multicenter group, 11 patients with AIMAH were systematically screened and were all found to have aberrant responses of cortisol secretion to at least one and often several stimuli [109]. In an *in vitro* screening study,

4 of 8 adrenals from AIMAH patients were found to express the GIP receptor in contrast to only 1 of 16 unilateral adenomas [66].

Two groups have reported the frequency of aberrant receptors in patients with AIMAH and subclinical CS. Four of 4 patients systematically screened with incidentally discovered AIMAH and biochemically confirmed subclinical CS were found to exhibit an aberrant receptor response: 2 combined V1-vasopressin receptor, LH/hCG receptor and 5-HT₄ receptor; 1 combined V1-vasopressin receptor and 5-HT₄ receptor, and 1 with V1-vasopressin receptor only [16]. Tatsuno et al. [110] reported 5 patients with AIMAH and subclinical CS who all showed a positive *in vivo* response to AVP. Overexpression of V1-AVP receptor mRNA by RT-PCR was shown in the 1 patient who was studied in more detail. Other aberrant responses were not systematically screened [110].

These studies convincingly suggest that most patients with AIMAH and CS, when systematically screened, have an aberrant stimulation of cortisol suggesting the expression of an illicit receptor in their adrenal glands. Based on these data and on the potential therapeutic implications of identifying an aberrant response (see later), we recommend that all patients with AIMAH and CS undergo screening as outlined by the screening protocol described above. The data for patients with AIMAH and subclinical CS are more scant and the screening protocol should be performed in the context of research protocols.

Molecular Mechanisms of Aberrant Receptors

The molecular mechanisms by which eutopic receptors and non-mutated ectopic receptors are expressed in the adrenal glands of patients with AIMAH and less commonly in adrenal adenomas are largely unknown. One may hypothesize that these aberrant receptors represent a primary phenomenon that is essential to the pathogenesis of AIMAH (resulting in both the hyperplasia and the hypercortisolism). Alternatively, this aberrant receptor expression may be an epiphenomenon as a result of cell proliferation and dedifferentiation. A number of observations would favor the former hypothesis including the presence of aberrant receptors in all patients with AIMAH who were systematically screened [83, 109], the *in vitro* stimulation of both hormonogenesis and cell division by the different implicated ligands [68], and the reversal of hyperplasia between pregnancies in LH/hCG-dependent CS [25]. Following this primary phenomenon, other genetic events may accumulate over time, as suggested by microarray data [35], that lead to additional proliferation signals and the formation of monoclonal nodules. This

mechanism is consistent with the finding of both monoclonal and polyclonal lesions within the same gland [78, 111–113].

The dysregulation of tissue-specific expression of membrane receptors implies either the disruption of *cis*-acting regulatory elements (promoters) or *trans*-acting elements (transcription factors/co-activators/co-repressors). This disruption must occur at an early stage of embryogenesis as the pathology involves the entire adrenal cortex. No mutations or polymorphisms were encountered in the promoter region of the GIP-receptor gene in GIP-dependent CS [114]. The identification of *trans*-acting elements implicated in tissue-specific expression of the hGIP receptor and their potential alterations are currently under way [115]. Alteration of *trans*-acting elements may be more likely and more consistent with the common co-expression of several aberrant receptors.

Kero et al. [116] have provided another interesting hypothesis. They demonstrated that chronic stimulation with LH in transgenic mice leads to adrenal CS as a result of LH/hCG receptor expression in the adrenal cortex. The expression of these ectopic receptors was thought to be the result of elevated estrogen levels as it was no longer observed following gonadectomy.

Management of AIMAH

Bilateral adrenalectomy is the treatment of choice in nearly all patients with AIMAH and CS. However, several case reports propose unilateral adrenalectomy as a safe and effective alternative [117]. A small series described 4 consecutive patients with AIMAH and CS in whom unilateral adrenalectomy was performed [118]. All patients experienced a clinical improvement and normalized their 24-hour urinary cortisol excretion but maintained an abnormal circadian rhythm and lack of suppression following dexamethasone administration. Their contralateral glands did not enlarge during 62–126 months of follow-up. However, the persistence of the subtle abnormalities of cortisol secretion in the subclinical CS range should cause concern as it has been shown to be associated with increased morbidity [119].

The identification of aberrant adrenal receptors provides the novel and interesting opportunity to treat selected patients with AIMAH and CS with pharmacologic agents. Octreotide administration was attempted in a few patients with GIP-dependent CS. The initial response to treatment was positive but long-term treatment proved to be ineffective [62, 67, 69]. In a patient with aberrant

β -adrenergic receptors and AIMAH, propranolol decreased cortisol secretion to approximately 2 times the upper limit of normal and was able to completely normalize cortisol secretion following only unilateral adrenalectomy [86]. Long-acting GnRH agonist treatment with leuprolide acetate has been shown to normalize cortisol secretion in a patient with AIMAH and LH-dependent CS as well as in a patient with LH-dependent subclinical CS [16]. The absence of adrenal insufficiency was hypothesized to be the result of the concomitant stimulation via the 5-HT₄ receptors. Goodarzi et al. [22] described a case of androgen-secreting AIMAH with an aberrant LH/hCG response in whom leuprolide acetate normalized androgen secretion. In contrast to the above successful treatment with a GnRH agonist, leuprolide acetate provided only a partial and transient decrease in cortisol production in another woman with aberrant responses to GnRH, LH, and hCG, suggesting the presence of unidentified aberrant receptors or post-receptor mechanisms [120]. A partial response to OPC-21268, a V1a-receptor antagonist, has been described in a patient with vasopressin-responsive CS [79]. These treatments have never been shown to reduce the size of the adrenal glands. The discovery of other aberrant receptors and the development of novel specific receptor antagonists will undoubtedly lead to a more widespread use of pharmacological manipulation for the treatment of AIMAH. It will also be of great interest to investigate whether treatment with these agents will alter the natural history of AIMAH when discovered in its subclinical stages.

The use of ketoconazole, an inhibitor of steroidogenesis, has been shown to be effective in restoring eucortisolism, especially in preparation for adrenalectomy [40, 121, 122]. The use of metyrapone and mitotane has also been reported in the literature in selected cases of AIMAH with CS [123].

In patients with subclinical CS, the decision to treat should be based on the age of the patient and the morbidity associated with the hypercortisolic state: hypertension, diabetes, osteoporosis, and neuropsychological manifestations. Follow-up with annual adrenal CT scan and biochemical assessment should be done in all these patients as the natural history of subclinical AIMAH is currently unknown. Taking into account recent reports of familial cases of AIMAH, it also appears justified to screen family members of patients with AIMAH for subclinical disease with overnight 1 mg dexamethasone test, late-night salivary cortisol, and adrenal CT scan.

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