Brief Review

Genetic and Potential Autoimmune Triggers of Primary Aldosteronism

Tracy Ann Williams, Paolo Mulatero, Martin Bidlingmaier, Felix Beuschlein, Martin Reincke

Hypertension is a major life-threatening disease of high morbidity and mortality, which affects between 10% to 40% of the general population in an age-dependent manner. The pathogenesis of essential hypertension is multifactorial, with various underlying contributory mechanisms. Primary aldosteronism (PA) is the leading cause of secondary hypertension that affects 4.3% of patients in the general hypertensive population and 9.5% of patients referred to hypertension units.1 The 2 predominant causes of the constitutive aldosterone secretion that define PA is either aldosterone-producing adenomas (APA) or bilateral adrenal hyperplasia (BAH, also called idiopathic hyperaldosteronism). Exome sequencing has unraveled the pathogenetic basis of around half of APA, but the underlying cause of the aldosterone excess in BAH remains an enigma.

The diagnosis of PA is made by a multistep process, recommended by the Endocrine Society guidelines,2 that ends with the invasive protocol of adrenal venous sampling. Once diagnosed, it is imperative to distinguish the surgically correctable forms (APA) from those that should be treated pharmacologically with mineralocorticoid receptor antagonists (BAH). With the application of wider screening for PA, >70% of cases of aldosterone excess have been defined as caused by BAH, with APA making up most of the remainder. APA and BAH are sporadic forms of PA, but 3 rarer familial forms of PA also exist called familial hyperaldosteronism (FH) types I, II, and III, described in more detail below.

Familial Hyperaldosteronism

Three familial forms of PA with distinct clinical characteristics have been described.3,4 FH type I or glucocorticoid remediable aldosteronism is caused by a hybrid gene resulting from the unequal crossing-over between the adjacent genes CYP11B1 and CYP11B2.5,6 Only few families (=50) have been identified worldwide. FH type II seems to be inherited as an autosomal dominant trait. A locus has been mapped on chromosome 7p22 in some but not all families,7 but the linkage area has not been resolved to any causative mutation. In addition, the candidate gene approach has been inconclusive, and the genetic basis of FH type II is still unknown. FH type III,8 however, has been linked to gain-of-function mutations in the potassium channel GIRK4.9 These mutations induce a loss in channel selectivity, depolarization, and increased intracellular Ca2+ concentrations leading to aldosterone excess. In a genetic analysis of 46 patients from 21 European families collected within the European FH type II consortium, a new germline G151E mutation was identified.10 Cells expressing the GIRK4 G151E mutation display a marked increase in Na+ conductance accompanied by Na+-dependent cell death, which may explain why patients carrying this mutation harbor no evidence of hyperplasia.11 Germline variants in the tumor suppressor gene ARMC5 associated with PA have also been reported recently, but the functional relevance of these variants was not studied.12 A clear familial form of PA has just been described that is caused by a heterozygous mutation in CACNA1H that encodes the voltage-gated calcium channel Cav3.2.13 The Cav3.2 M1549V mutation results in a dramatic impairment of channel inactivation and activation at more hyperpolarized potentials resulting in an increase in intracellular Ca2+ concentrations.13

Sporadic PA

The application of next-generation sequencing methods during the past 3 years has resulted in the identification of genetic contributors to the endocrine autonomy of APAs, such as the definition of point mutations in the potassium channel GIRK4 (encoded by KCNJ5) in 34% to 36% of adenomas.3,14 Somatic APA mutations in members of the ATPase family (Na/K+-ATPase 1 and Ca2+-ATPase 3, encoded by ATP1A1 and ATP2B3, respectively)15–17 and in a subunit of an L-type voltage-gated Ca2+-channel, Cav1.3 (encoded by CACNA1D), were subsequently identified.16,18 Together, mutations in these genes explain the constitutive aldosterone production in ≈50% of cases as demonstrated recently in a large European cohort comprising 474 APAs, recruited through the European Network for the Study of Adrenal Tumors registry (http://www.ensat.org/).19 The majority of the GIRK4-APA mutations lie in or within the close proximity of the ion selectivity filter of the K+ channel and result in the indiscriminate conductance of Na+ that causes membrane depolarization, Ca2+ influx, and increased aldosterone biosynthesis.5 Mutations in the Na+/K+-ATPase 1 produce a decrease in K+ binding that results in the
reduced import of K⁺ and export of Na⁺ and also causes cell depolarization.15,17 This in turn results in the opening of voltage-gated Ca²⁺-channels. In contrast, the Ca²⁺-ATPase mutations were proposed to affect the clearance of cytoplasmatic calcium ions.15 The net result of mutations in both ATPases is therefore likely to cause an increase in the intracellular Ca²⁺ concentration and as a consequence, an upregulation of aldosterone biosynthesis.15 The Cav1.3 mutations (CACNA1D) have been reported to result in channel activation at less depolarized potentials and cause calcium influx and aldosterone production.18

These developments present a major breakthrough in the field and highlight the power of current state-of-the-art genetic and molecular tools. In sharp contrast, the pathophysiology of BAH remains obscure. Its apparent phenotypic variability in clinical, biochemical, and morphological aspects suggests that BAH may not be a distinct entity but rather represents the variable response of the adrenal cortex to genetic, environmental, or humoral factors.

**Autoantibodies in PA**

A role for circulating factors in PA has long been postulated. Recently, stimulating antibodies directed against the angiotensin II type 1 receptor (AT₁R) have been identified in the serum of patients with PA leading to the hypothesis that these autoantibodies may contribute to putatively cause and sustain aldosterone excess in some of these patients. AT₁R autoantibodies (AT₁-AA) were first detected in the serum of patients with PA by Rossitto et al.20 These authors demonstrated that AT₁-AA were detected in 92% of patients with APA at a 2-fold higher titer compared with patients with BAH, despite similar blood pressure levels, and hypothesized that they could have a future use in differentiating these 2 PA subtypes (Table). The agonistic nature of the AT₁-AA isolated from patients with PA to the AT₁R was subsequently demonstrated where they were shown to exhibit losartan- and candesartan-sensitive AT₁R activation in a cell-based functional assay.21 Furthermore, the AT₁-AA induced small resistance artery contraction and stimulated aldosterone production in cultured adrenal cells. The AT₁-AA–mediated production of aldosterone in the adrenal cell line was enhanced in the presence of low doses of angiotensin II, suggesting that the antibodies may alter the allosteric configuration of the AT₁R and facilitate angiotensin II binding.21 A follow-up study by the same group on a larger series of patients with PA demonstrated an increased prevalence in BAH compared with those with an APA (75% versus 46%) (Table). This predominance of the AT₁-AA in the BAH subtype rather than the APA subtype is in direct contrast to the original findings.20 Moreover, the APA and BAH subtypes with contrasting AT₁-AA titers displayed similar blood pressure levels in 1 study,26 whereas Li et al23 described a modest correlation of AT₁-AA functional activity with mean arterial pressure. However, the 2 studies used different assays to measure AT₁-AA. Rossitto et al20 used an ELISA-based assay, whereas Kem et al23 and Li et al22 used cell-based functional assays; in fact, in a direct comparison, the 2 types of assay give widely different results.23 However, the inconsistency surrounding the prevalence of AT₁-AA in patients with PA should be resolved using large cohorts of patients with PA who have been accurately subtyped according to strict criteria23 and using extensive controls including patients with preeclampsia as positive controls in reliable functional assays. The reported increased prevalence of AT₁-AA in patients with BAH is of potential relevance to the distinct phenotypes of adrenal sensitivity to angiotensin II of patients with BAH and APA.

Patients with BAH are more responsive to angiotensin II infusion than most of those with APA, and in addition, after overnight recumbency, they display an increase in plasma aldosterone on standing.24,25 Conceivably, AT₁-AA at a higher titer in patients with BAH could contribute to such a distinction if they were to act as positive allosteric modulators and enhance the binding affinity of the receptor for its cognate ligand. Because patients with low renin primary hypertension also display an increased responsiveness to angiotensin II,24 this has led some authors to suggest that these patients could also harbor increased titers of AT₁-AA.25

AT₁-AA could be a key factor in the chronic stimulation of the zona glomerulosa. The resulting hyperproliferative state could set the stage for an increased susceptibility to somatic gain-of-function mutations that give rise to APA. In line with such a scenario is the observation of an often hyperplastic adrenal cortex adjacent to the APA26 despite suppressed renin concentrations. The possible relationship of AT₁-AA with APA mutational status has to be clarified but could yet prove to be the missing puzzle piece in the multistep process of the pathogenesis of PA and provide the link between BAH and PA. Indeed, PA may join the expanding list of hypertensive and cardiovascular disorders in which humoral-mediated autoimmune mechanisms may play a pathological role. Autoimmune responses have long been known to trigger or exacerbate a variety of different diseases and result

**Table. Prevalence of Angiotensin II Type 1 Receptor Activating Antibodies in Hypertensive Disorders**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>No. of Study Subjects</th>
<th>Prevalence</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preeclampsia</td>
<td>25</td>
<td>100%</td>
<td>Wallukat et al40</td>
</tr>
<tr>
<td>16</td>
<td>100%</td>
<td>Thway et al54</td>
<td></td>
</tr>
<tr>
<td>5 and 19</td>
<td>80 and 89%*</td>
<td>Walthier et al41</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>81%</td>
<td>Yang et al43</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>70%</td>
<td>Herse et al46</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>95%</td>
<td>Siddiqui et al45</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>48%</td>
<td>Zhang et al42</td>
<td></td>
</tr>
<tr>
<td>Renal-allograft rejection and malignant hypertension</td>
<td>16</td>
<td>100%</td>
<td>Dragun et al52</td>
</tr>
</tbody>
</table>

APA indicates aldosterone-producing adenoma; and BAH, bilateral adrenal hyperplasia.

*80% and 89% in second and third trimesters of pregnancy, respectively.
from abnormal B- and T-cell recognition of self-antigens that can lead to the production of autoantibodies by B-cell derived plasma cells. Hence, B cells are considered as the primary contributors to the pathogenesis of an autoimmune disease via their role in autoantibody production. Curiously, an increasing number of autoimmune diseases are reported to involve autoantibodies that target G-protein–coupled receptors. An established example of this is Graves disease in which stimulatory autoantibodies to the thyroid hormone receptor lead to hyperthyroidism. In one of the first demonstrations of antibody transfer causing autoimmune disease, 4 authors of the study by Adams et al established that thyroid gland stimulation resulted from self-infusion of plasma from patients with Graves disease. In addition to agonistic autoantibodies that induce thyroid hormone synthesis, secretion, and thyroid cell proliferation, thyroid hormone receptor autoantibodies have been described that are blocking or neutral. Some of the blocking autoantibodies that ablate thyroid hormone action cause hypothyroidism. In an apparent paradox, some blocking and also some neutral autoantibodies have been shown to elicit distinct signaling pathways and cellular responses in vitro.

However, many diseases triggered by an immune response against G-protein–coupled receptors involve disorders of the cardiovascular system. Notable examples involve autoantibody responses to the β1-adrenergic receptor in dilated cardiomyopathy and to the α1-adrenergic receptor in different forms of hypertension. Autoantibodies against the AT1R in pre-eclampsia and in renal-allograft rejection, particularly associated with malignant hypertension, have also been reported. The potential role of humoral-mediated immune responses in hypertensive and cardiovascular disorders is outlined in more detail below.

### Autoantibodies in Hypertension and Cardiovascular Disorders

#### Autoantibodies to Adrenergic Receptors

Autoantibodies against the β1-adrenergic receptor are associated with dilated cardiomyopathy and are present in ≥40% of affected patients. Their central role in this disorder was demonstrated by the immunization of rats against the second extracellular β-adrenoceptor loop and the transfer of serum from these animals to nonimmunized rats that resulted, in both sets of animals, in the induction of left ventricular dilation and dysfunction. Furthermore, β1-adrenergic receptor autoantibodies are an independent predictor of sudden cardiac death and are associated with a 3-fold increased risk of all-cause and cardiovascular mortality.

**Immune responses to the α1-adrenergic receptor are associated with different forms of hypertension.** Thus, 20% of patients with malignant hypertension, 44% of patients with primary hypertension, and 51% of patients with refractory hypertension have been shown to harbor agonistic α1-adrenergic receptor autoantibodies. A functional role of α1-adrenergic receptor autoantibodies was demonstrated by immunoadsorption, which successfully decreased the mean arterial blood pressure in a group of 5 patients with refractory hypertension.

**Autoantibodies to the AT1R in Preeclampsia**

Preeclampsia is a serious disorder of pregnancy that is characterized by hypertension, proteinuria, and other systemic disturbances and is the leading cause of induced preterm delivery worldwide. Despite being a frequent complication that affects 2.5 to 3.0% of pregnancies, the pathogenesis of this disorder remains undefined and an effective treatment is unavailable. The possibility that preeclampsia could be an autoimmune disease was first raised by Wallukat et al who elegantly demonstrated that patients with preeclampsia develop autoantibodies to AT1- AA and were absent from pregnant patients who were either essential hypertensives or normotensives. Functional activity of AT1-AA was demonstrated in a cardiomyocyte contraction assay, and specificity was established with the AT1 antagonist losartan and with a synthetic peptide corresponding to a segment of the AT1R second extracellular loop (AFHYESQ, position 165–191). A multitude of studies subsequently both confirmed and advanced these findings. AT1-AA are highly prevalent in preeclampsia (Table); they are detected as early as 18 weeks of pregnancy, and their levels vary according to trimester and fall after delivery although they do not regress completely persisting in 20% of cases 1 year post partum. Comparison of AT1-AA levels in patients with mild and severe preeclampsia demonstrated a strong correlation of autoantibody titer and severity of the disease notably in terms of hypertension and proteinuria.

Zhou et al performed a key study that provided strong evidence for preeclampsia being a pregnancy-induced autoimmune disease. This study demonstrated that injection of activating AT1-AA, purified from women with preeclampsia, into pregnant mice induced key features of preeclampsia including hypertension and proteinuria that were abolished by coinjection with losartan or the AFHYESQ peptide (Figure). Moreover, infusion of AT1-AA into pregnant rats induced a hypertensive response that seemed to be via an endothelin-1–dependent mechanism. In another study, injection of AT1-AA into pregnant rats induced symptoms consistent with preeclampsia, including hypertension, proteinuria, and intrauterine growth retardation that was associated with the upregulation of hypoxia-inducible factor-1α and endothelin-1. In this case, however, the phenotypic effect was only observed when the antibody transfer was performed in combination with angiotensin II, that is, not when transferred alone as in other studies.

Therefore, a wealth of studies have demonstrated a key role for AT1-AA in the pathophysiology of preeclampsia. The efficacy of the AFHYESQ epitope–blocking peptide in abolishing the AT1-AA functional response has been established, thus raising the possibility of epitope peptide therapy as a tenable therapeutic strategy to treat preeclampsia.

#### Autoantibodies to the AT1R in Renal-Allograft Rejection and Malignant Hypertension

Allograft rejection is classically thought to be mediated by T cells; however, a growing body of evidence supports an important role for antibody-mediated rejection (humoral or vascular rejection) that is now thought to account for 30% to 35% of acute episodes of kidney graft failure. Vascular
rejection is associated with high titers of antibodies against human leukocyte antigens, but non–human leukocyte antigen antibodies can also be pathogenic drivers, and in these cases, the patients are particularly difficult to treat. In 2005, Dragan et al reported the presence of AT1-AA and the absence of anti–human leukocyte antigen antibodies, in all 16 of their patients with renal-allograft rejection who had also developed hypertension before vascular rejection was absent in 13 of the 16 patients, thereby indicating that the hypertension was secondary to the rejection. It was the clinical profile of the first study patient, reminiscent of a preeclampsia crisis accompanied by seizures, that led the authors to study the role of AT1-AA. Later studies confirmed that AT1-AA display a significant association with renal graft failure and that they are an independent predictor of graft rejection fascinatingly, not yet be revealed as a missing piece of a puzzle that is being rapidly solved.

**Conclusions**

Functionally active autoantibodies against G-protein–coupled receptors have been discovered in the sera of patients with many different cardiovascular disorders acting in several cases as pathogenic drivers. Some of these involve autoantibodies against AT1R that share a common theme in that they recognize epitopes in the second extracellular loop of the receptor as for the cognate ligand. However, the factor that elicits the production of AT1-AA is unknown. AT1R itself could plausibly be a trigger or, as has been described in some humoral-mediated autoimmune diseases, a defect associated with CD40 with its role in B-cell activation. Moreover, the molecular mechanisms whereby AT1-AA activate AT1R remain to be elucidated, and the signaling pathways elicited and their associated cellular responses still have to be characterized. Nonetheless, AT1-AA have a defined role in preeclampsia and realistic therapeutic strategies aimed at their removal by plasmapheresis or by abolishing AT1-AA function with an epitope-blocking peptide have been proposed. There is a striking link between AT1-AA and renal-allograft rejection with malignant hypertension, and these examples together with other autoimmune mechanisms involving the α- and β-adrenergic receptors that are associated with hypertensive disorders raise the fascinating concept of autoimmune mechanisms in hypertension. The role of autoantibodies in PA requires some clarification, but AT1-AA may yet be revealed as a missing piece of a puzzle that is being rapidly solved.

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**Disclosures**

None.

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