Primary aldosteronism (PA) is the most common form of secondary hypertension, with an estimated prevalence of \( \approx 10\% \) in referred patients and 4\% in primary care\(^1,2\) but as high as 20\% in patients with resistant hypertension.\(^3,4\) It is characterized by hypertension with low plasma renin and elevated aldosterone that is often seen with hypokalemia. PA occurs as a result of a dysregulation of the normal mechanisms controlling adrenal aldosterone production. The 2 major causes are aldosterone-producing adenomas (APAs) and bilateral adrenal hyperplasia (BAH), also called idiopathic hyperaldosteronism. The early detection of PA has an enormous impact on clinical outcome and survival, given the major cardiovascular adverse effects of aldosterone excess, which are independent of blood pressure.\(^5,6\) and predict outcome after surgical treatment.\(^7,8\) Patients with PA have a significantly higher risk of nonfatal myocardial infarction, atrial fibrillation, and stroke compared with age-, sex-, and blood pressure–matched essential hypertensives.\(^5\) Furthermore, changes in cardiac structure and function\(^9\) and partially reversible renal dysfunction\(^10\) have been described, whereas the increased occurrence of metabolic abnormalities in patients with PA is still a matter of debate.\(^5,11,12\)

Although the 2008 guidelines for the management of PA have been pivotal for homogenizing screening procedures and treatment among specialized centers,\(^13\) there remain a few critical issues related to diagnosis, subtype differentiation, and treatment of nonsurgically correctable forms. Reliable diagnostic and prognostic biomarkers are lacking for more sensitive and specific screening, as well as new therapeutical avenues, because medical and/or surgical treatment of PA leads to normotension in only a minority of patients. This may come from a better understanding of the pathogenic mechanisms of the disease, in particular, identification of the genetic and molecular determinants leading to the development of APA and BAH. Over the last few years, considerable efforts have been made to this end by different groups, taking advantage of developments in high-throughput “omics” technologies, in particular transcriptomics, and more recently new generation exome sequencing, together with genetically modified mouse models. This review summarizes our current knowledge on the genetics (genes and their roles in inheritance) and genomics (genome-wide studies addressing variations in gene structure and expression) of PA, in both familial and sporadic forms of the disease, trying to integrate genetic abnormalities and gene dosage effects into a pathophysiological perspective related to aldosterone production and cell proliferation.

**Genetic Forms of PA**

Although the majority of PA is apparently sporadic, 3 familial forms displaying mendelian inheritance have been described. Familial hyperaldosteronism (FH) 1, also called glucocorticoid-suppressible aldosteronism, was first described \( \approx 50 \) years ago.\(^14\) The disorder is characterized by early and severe hypertension, most often before the age of 20 years, in subjects with biochemical abnormalities of PA of variable intensity and in some cases adrenal nodules.\(^15\) FH-1 is inherited as an autosomal dominant trait and is characterized by significant production of hybrid steroids (18-hydroxycortisol and 18-oxocortisol) and normalization of aldosterone levels and blood pressure with low doses of dexamethasone.\(^16\) The condition reflects the presence of a hybrid gene resulting from unequal crossing over between the adjacent highly homologous genes \( CYP11B2 \) and \( CYP11B1 \) on chromosome 8q21-q22, coding for aldosterone synthase and steroid 11\(\beta\)-hydroxylase.\(^17,18\) Fusion of the promoter region of \( CYP11B1 \) to the coding region of \( CYP11B2 \) produces a chimeric gene, with activity of aldosterone synthase, but tissue specificity and regulation that of 11\(\beta\)-hydroxylase, so that the synthesis of aldosterone is under control of adrenocorticotropic hormone rather than plasma potassium concentrations and the renin-angiotensin system. Although the prevalence of FH-1 in patients with PA has been reported previously to be \( \approx 0.36\% \),\(^19\) more recent studies, including large cohorts of patients with PA, set the prevalence of FH-1 between 0.66\% and 1.00\%.\(^20,21\) Prevalence as high as 3.1\% has been reported recently in a hypertensive pediatric population.\(^22\)

FH-2 also reflects autosomal dominant transmission, but not associated with a hybrid gene and with hyperaldosteronism not suppressible by dexamethasone.\(^23\) The phenotype is variable from APA to BAH, with a variable response of
aldosterone to angiotensin II (Ang II). Clinical and biological features of FH-2 are indistinguishable from sporadic PA, and FH-2 is diagnosed on the basis of ≥2 affected members in a family, however, with phenotypic variability within affected families typical for the disease. The prevalence of FH-2 is estimated to be between 2.8% and 6.0% in adult populations with PA. In a large family with FH-2, a locus associated with the disease has been mapped to chromosome 7p22, although no causal mutations have been identified in any of the genes located in the linkage area thus far. We have explored in depth the family history of 350 index cases in our PA database. In this study, 12 families (4%) had criteria suggestive of FH-2, with ≥2 affected first-degree relatives. The distribution of the affected subjects within the families was compatible with an autosomal dominant trait, with a high number of hypertensives in additional family members, suggesting the possibility of other unrecognized cases. Analysis of positional candidates located at chromosome 7p22 showed no mutations on the genes coding for fascin 1 (FSCN1) and the cAMP-dependent protein kinase type 1-β regulatory subunit (PRKAR1B).

Candidate gene approaches to study the genetic basis of FH-2 and sporadic PA have included genes involved in steroidogenesis or in familial cancer syndromes. In some tumors, loss of heterozygosity was found at chromosome 11q13 harboring the MEN1 locus, which is a tumor suppressor gene responsible for multiple endocrine neoplasia type 1, characterized by adenomas of the adrenal cortex in 30% of cases. In patients experiencing FH-2, as well as in patients with sporadic APA, no genetic abnormality has been identified in genes coding for aldosterone synthase (CYP11B2), the Ang II type 1 receptor (AT1R), or the tumor suppressor gene p53 (Reference 28 and references therein). However, a higher percentage of a particular allele of the CYP11B2 gene (~344T) was found in hypertensive patients with elevated aldosterone to renin ratio, which is compatible with PA.

More recently, a third form of familial hyperaldosteronism, referred to as FH-3, was reported in a family with severe, early-onset hypertension resistant to treatment. Affected family members showed hyporeninemia, hyperaldosteronism, and very high levels of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol but no suppression of aldosterone production by dexamethasone. Early bilateral adrenalectomy was required to correct blood pressure, showing dramatic enlargement of the adrenals with massive hyperplasia of the cortex. The genetic basis of FH-3 has been elucidated recently (see below).

### From Physiological to Pathological Aldosterone Production

In the adrenal cortex, cortisol and aldosterone are synthesized by the isozymes steroid 11β-hydroxylase (encoded by CYP11B1) and aldosterone synthase (encoded by CYP11B2), respectively. 11β-Hydroxylase catalyzes the 11β-hydroxylation of 11-deoxycortisol to cortisol and of 11-deoxycorticosterone to corticosterone. Aldosterone synthase catalyzes the 11β-hydroxylation of 11-deoxycorticosterone to corticosterone, 18-hydroxylation of corticosterone to 18-hydroxycorticosterone, and 18-oxidation of 18-hydroxycorticosterone to aldosterone in the zona glomerulosa (ZG). Aldosterone production is tightly controlled to maintain electrolyte and fluid homeostasis by the kidney. Thus, the 2 most important physiological stimuli of aldosterone secretion are Ang II and serum potassium. The key player in regulating aldosterone biosynthesis is calcium (Ca2+) signaling. Stimulation of glomerulosa cells by Ang II or potassium results in depolarization of the ZG cell membrane and opening of voltage-dependent Ca2+ channels, leading to an increased intracellular Ca2+ concentration. Ang II also signals through the Ang II type 1 receptors to stimulate inositol phospholipid turnover, activating of the calcium signaling pathway triggers a phosphorylation cascade, involving calmodulin and calmodulin-dependent kinase II/IV, ultimately leading to the activation of transcription factors (ie, Nur1 and NGF1B) that positively regulate the transcription of CYP11B2.

Different studies have explored gene expression changes in APA, investigating relevant candidate genes and more recently using pangenomic approaches. Among the candidate genes, those coding for steroidogenic enzymes in the adrenal gland have been intensively investigated (Table 1). Using various techniques, several studies have thus reported increased expression of CYP11B2 in APA compared with normal adrenals or peritumoral adjacent cortex (throughout this review, “adjacent cortex” refers to the entire cortex of an adrenal gland harboring an APA). In addition to CYP11B2, expression of CYP21A2 (coding for steroid 21-hydroxylase, responsible for the conversion of progesterone into 11-deoxycorticosterone) has been found to be consistently increased in APA, whereas mRNA levels of CYP11B1 were decreased and there were no changes in CYP11A1 expression.

Remarkably enough, these studies also revealed a relative heterogeneity in terms of gene expression. In particular, HSD3B2 (encoding 3β-hydroxysteroid dehydrogenase responsible for the conversion of pregnenolone into progester-
one) has been found to be either increased\textsuperscript{57} or unchanged \textsuperscript{58} whereas expression of CYP17A1 (coding for the steroid 17α-hydroxylase/17,20 lyase responsible for the conversion of pregnenolone and progesterone to the respective 17α-hydroxylated precursors of glucocorticoids and adrenal androgens) was either decreased\textsuperscript{36,38} or unchanged.\textsuperscript{32} Most unexpectedly, however, different authors found subsets of tumors with highly heterogeneous expression of CYP11B2 either increased, unchanged, or decreased compared with control adrenal tissue.\textsuperscript{34,36} A very recent transcriptome study further demonstrated the tumor heterogeneity of CYP11B2 expression and the correlation of high versus low CYP17A1 expression in APA with morphological features, that is, adenomas composed of zona fasciculata–like cells versus tumors composed of ZG-like cells, respectively.\textsuperscript{39}

One possible reason for different studies reporting varying results in terms of the heterogeneity of CYP11B2 expression in APA samples may relate to the small number of samples investigated, the different technical approaches used, and/or the different screening procedures for selecting patients for surgery. In addition, in some studies APAs were compared with adrenal cortices obtained from nephroarenelectomies for renal cancer, whereas others used adjacent adrenal cortex as a control, with both tissues having their own pitfalls (see below). An additional reason, however, is most probably related to the real heterogeneity of the molecular phenotype of APA, reflecting the possibility that modifications in different pathways may converge to increase aldosterone production and cell proliferation. This is supported by 2 subsequent transcriptome analyses performed in our laboratory on 123 APA samples and 11 control adrenals, which showed a subgroup structure that could reflect distinct pathogenic mechanisms and which was confirmed by functional pathological investigations.\textsuperscript{40,41} Nevertheless, CYP11B2 expression appears to be consistently increased, although to variable extent, among APA samples, with low expression of CYP11B2 in APA being a rare finding.\textsuperscript{42}

### Intracellular Signaling and Transcriptional Regulation

In response to the intracellular increase in $\text{Ca}^{2+}$ concentration induced by Ang II and $\text{K}^+$, the mitochondria rapidly take up $\text{Ca}^{2+}$, and a slower activation of calcium/calmodulin dependent kinase is observed. The mitochondria play a prominent role in $\text{Ca}^{2+}$ homeostasis by capturing $\text{Ca}^{2+}$ when its concentration rises in the cytosol. The rate-limiting step in steroid biosynthesis is the conversion of cholesterol to pregnenolone, which occurs in the mitochondria and requires the transfer of the substrate from the cytosol across the double membrane. The rise in intracellular $\text{Ca}^{2+}$ stimulates both intramitochondrial cholesterol transfer and the expression of the steroidogenic acute regulatory protein StAR, which promotes the transport of cholesterol from the outer to the inner mitochondrial membrane and constitutes the rate-limiting step in steroid hormone biosynthesis.\textsuperscript{43} Despite its critical role, the expression of StAR shows only small variations among the different types of adrenal tumors, including APA, cortisol-producing adenomas, and nonfunctional adenomas, compared with normal adrenal.\textsuperscript{37,44} Overexpression of the high-density lipoprotein scavenger receptor B1 suggests that cholesterol supply to the cell is increased through the high-density lipoprotein pathway; increases in adrenodoxin and P450 oxidoreductase indicate that additional electrons are provided via these 2 pathways to both mitochondrial P450 enzymes and enzymes located in the endoplasmic reticulum\textsuperscript{38} (Table 1).

Assié et al\textsuperscript{39} showed that several genes involved in calcium signaling were differently expressed between control adrenal and APA, particularly the calcium effector calmodulin and genes encoding proteins involved in calcium storage, that is, the endoplasmic reticulum calreticulin and the calcium ATPase SERCA3. These results indicate an increase in calcium signaling and in intracellular calcium tone. One explanation for the activation of calcium signaling in APA may be an abnormal expression of G-protein coupled receptors triggering chronic calcium activation as one of the initial and critical events responsible for the development of PA. Indeed, in adrenal Cushing syndrome, ectopic peptide hormone receptor expression has been demonstrated as the basis for the excessive glucocorticoid secretion in a subset of patients.\textsuperscript{45} Several analyses demonstrate ectopic expression of G-protein coupled receptors in some APAs (Table 2), including the 5-HT receptor 4,\textsuperscript{46,47} the adrenocorticotropic

### Table 2. Calcium Signaling and Transcriptional Regulation in APA

<table>
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<tr>
<th>Gene Name (Protein)</th>
<th>Expression in APA</th>
<th>References</th>
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<tr>
<td>CALM2 (calmodulin)</td>
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<td>38</td>
</tr>
<tr>
<td>ATP2A3 (SERCA3)</td>
<td>↑</td>
<td>38</td>
</tr>
<tr>
<td>G protein–coupled receptors</td>
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<td></td>
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<tr>
<td>HTR4 (5-HT4)</td>
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<tr>
<td>MC2R (ACTH-R)</td>
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<td>47, 48</td>
</tr>
<tr>
<td>LHCGR (LHR)</td>
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<td>47, 49</td>
</tr>
<tr>
<td>HTR7 (5-HT7)</td>
<td>↑</td>
<td>47, 50</td>
</tr>
<tr>
<td>ATR1 (AT1, R)</td>
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</tr>
<tr>
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<td>54</td>
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<tr>
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<td>47</td>
</tr>
<tr>
<td>GRP37 (endothelin B receptor-like protein 1)</td>
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<td>47</td>
</tr>
<tr>
<td>GRM3 (metabotropic glutamate receptor 3)</td>
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Nerve receptors and transcription factors

<table>
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<th>Expression in APA</th>
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<td>↑ or →</td>
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<tr>
<td>NR0B1 (OX-1)</td>
<td>↑ or →</td>
<td>36, 37</td>
</tr>
<tr>
<td>GATA4 (transcription factor GATA-4)</td>
<td>↑</td>
<td>37</td>
</tr>
<tr>
<td>GATA6 (transcription factor GATA-6)</td>
<td>↑</td>
<td>37</td>
</tr>
</tbody>
</table>

NGF1B indicates nerve growth factor 1B; SF-1, steroidogenic factor 1; DAX-1, dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1; 5-HT, 5-hydroxytryptamine receptor 4; ACTH-R, adrenocorticotropic hormone receptor; LHR, luteinizing hormone receptor; 5-HT7, 5-hydroxytryptamine receptor 7; AT1, type 1 angiotensin II receptor; GnRH-R, gonadotropin releasing hormone receptor; SERCA3, sarcoplasmic/endoplasmic reticulum calcium ATPase 3; ↑, increased expression; →, unchanged expression; ↓, decreased expression; APA, aldosterone-producing adenoma.
hormone receptor (MC2-R),\textsuperscript{37,48} the luteinizing hormone receptor (LHR),\textsuperscript{47,49} or the 5-HT7 receptor.\textsuperscript{47,50} It is interesting to note that, in vitro, serotonin (5-HT) is able to stimulate aldosterone production through the activation of the 5-HT receptor 4.\textsuperscript{51} Overexpression of the Ang II type 1 receptor\textsuperscript{52,53} and downregulation of the dopamine D2 receptor have also been described.\textsuperscript{54} In a subset of APAs, overexpression of the putative endothelin receptor type-like protein (GPR37), the glutamate receptor metabotropic 3 (GRM3), and the gonadotropin-releasing hormone receptor (GNRHR) has also been described.\textsuperscript{47} All of these receptors are coupled to different G proteins (G\textsubscript{i}, G\textsubscript{i1}, G\textsubscript{q}, or G\textsubscript{i2/G13}), potentially leading to activation of different signaling pathways in APAs.

The regulation of steroid biosynthetic enzymes is under the control of several orphan nuclear receptors and transcription factors. The presence of specific response elements has been reported on the human CYP11B2 promoter, in particular, a cAMP response element, also called adrenal 1, a nerve growth factor-induced clone B (NGFIB) response element (NBRE-1), and Ad4 and Ad5 elements.\textsuperscript{55} Interestingly, NBRE-1 and Ad5 were found in the 5'-flanking region of CYP11B2 but not of CYP11B1. The NBRE-1 site is a nuclear receptor half-site that binds NGFIB family members, which includes NGFIB (NR4A1) and NURR1 (NR4A2). The Ad5 element represents a direct repeat of 2 nuclear receptor half-sites in tandem, which can bind NGFIB family members SF-1 (NR5A1) and COUP-TF (chicken ovalbumin upstream promoter-transcription factor), whereas the Ad4 sequence is bound specifically by SF-1, the main transcription factor involved in steroidogenesis. SF-1 and DAX-1 are 2 orphan nuclear receptors known to regulate STAR, CYP11A, and CYP17A1. Given that DAX-1 is a known repressor of SF-1, it has been suggested that, in addition to the level of expression of these receptors, it is the SF-1/DAX-1 ratio that could be important in regulating adrenal steroid hormone production.\textsuperscript{55} Although the expression of genes coding for NURR1, SF-1, and DAX-1 has been reported to be increased or unchanged in APA, investigations into the expression of GATA4 and GATA6, coding for transcription factors involved in adrenal cortisol and androgen production through the regulation of CYP17A1 and/or steroid sulfotransferase 2A1 (SULT2A1), show increased expression of both\textsuperscript{57} (Table 2). It might well be that, more than modification of expression of these transcription factors, the nonappropriate activation of G protein--coupled receptors could modulate their phosphorylation state through different signaling pathways, thus leading to binding to specific target sequences and activation of gene transcription.

Potassium Channels and the New Era of PA

Among other potassium channels, TWIK-related acid sensitive potassium (TASK) channels are highly expressed in the adrenal cortex and are central to the maintenance of the membrane resting potential of ZG cells.\textsuperscript{56} The functional relevance of TASK channels has been highlighted by 2 different mouse models both developing hyperaldosteronism, albeit with important pathophysiological differences. Genetic deletion of Task1 led to depolarization of adrenocortical cells and to a remarkable ectopic expression of aldosterone synthesis in the zona fasciculata of female mice. As a result, female Task1\textsuperscript{--/--} mice display a diet-independent, low renin, and glucocorticoid-remediable form of hyperaldosteronism.\textsuperscript{57} Neonatal male mice show the same features as females but at puberty become normal in terms of zonation and steroid secretion. In Task1/Task3 double knockout male mice, glomerulosa cells showed no acid-sensitive K\textsuperscript{+} current and were strongly depolarized. The mice showed low renin hyperaldosteronism partly responsive to sodium diet, with a phenotype reminiscent of patients with idiopathic primary hyperaldosteronism.\textsuperscript{58} Although these models suggest that hypomorphic alleles of the KCNK3 and KCNK9 genes, coding for TASK1 and TASK3, respectively, may play a role in the development of PA in humans, sequencing of these genes in 22 patients with APA,\textsuperscript{59} as well as in both germline and somatic DNA from a large cohort of patients with PA in our laboratory,\textsuperscript{60} has not led to the identification of causative mutations.

A major advance in our understanding of the pathogenesis of PA has recently come from the identification of recurrent somatic mutations of the KCNJ5 gene in APA, as well as germline mutations of the same gene in families with FH-3.\textsuperscript{40,59,61-63} KCNJ5 encodes the G protein–activated inward rectifier potassium channel Kir3.4. The different mutations identified in APA (p.G151R and p.L168R) and FH-3 (p.T158A) are all located near or within the selectivity filter of Kir3.4 and affect the ion selectivity of the channel, with increased sodium conductance leading to chronic membrane depolarization. These changes are presumed to be responsible for the constitutive secretion of aldosterone and for cell proliferation attributed to the continued opening of membrane voltage-dependent calcium channels and activation of the calcium signaling pathway.\textsuperscript{59} The causal link to aldosterone production has been formally established by Oki et al.,\textsuperscript{64} who demonstrated that expression of channels harboring the inherited p.T158A mutation in adrenal cortical carcinoma cells potentiated aldosterone production both in basal conditions and after stimulation by Ang II. The increase in aldosterone biosynthesis was dependent on membrane depolarization followed by calcium influx leading to increased expression of CYP11B2. In contrast, it remains unclear whether or how KCNJ5 mutations affect cell proliferation, because expression of mutated channels in 2 different cell lines resulted in either reduced cell proliferation\textsuperscript{64} or rapid sodium-dependent cell lethality.\textsuperscript{65}

Analysis of a large number of patients (380 APA and 174 BAH), recruited through referral centers from the European Network for the Study of Adrenal Tumors (ENS@T, www.ensat.org), has shown that somatic KCNJ5 mutations are present in a large proportion of APAs, with an estimated prevalence in unselected patients of 34%.\textsuperscript{40} Even higher frequencies have been described, depending on sample size, screening procedures for selecting patients for adrenalectomy, and genetic background.\textsuperscript{62,63} In contrast, germline mutations of the KCNJ5 gene are not similarly responsible for sporadic BAH, the most common form of PA in Western countries.\textsuperscript{40} KCNJ5 mutations in APAs appear to be more prevalent in females than males and in younger patients and are associated with higher preoperative aldosterone levels but not with therapeutic outcomes after surgery.\textsuperscript{40} Although an
association of KCNJ5 mutations with tumor size has been described in 1 study investigating 73 patients with APA.\textsuperscript{62} This result has not been replicated in our large cohort of unselected patients\textsuperscript{40} or in a recent study from Japan.\textsuperscript{63}

Interestingly, a significant association of genotype with the aldosterone response to upright posture (corresponding to the rise in plasma aldosterone from the recumbent to the upright position) has been reported in 10 patients.\textsuperscript{62}

Following the first description of a p.T158A KCNJ5 mutation in a severe form of FH3, additional mutations and phenotypic variability of FH3 have been reported. In particular, the same p.G151R mutation occurring as a somatic event in APA was found as an inherited mutation in 2 families with early and severe hyperaldosteronism. These patients had bilateral adrenal cortical hyperplasia and required bilateral adrenalectomy during childhood to control blood pressure.\textsuperscript{59}

Conversely, a second inherited mutation affecting the same amino acid, p.G151E, was described recently in 3 families with a diagnosis of nonglucocorticoid remediable familial hyperaldosteronism.\textsuperscript{61,65} Remarkably, although this mutation had similar consequences on channel function as the previously described mutations, it was associated with a much less severe phenotype, resembling that of FH-2, with blood pressure and hypokalemia easily controlled by medication and no evidence of adrenal hyperplasia.

**New Signaling Pathways Contributing to APA Formation**

Using once again a transcriptomic approach, Williams et al\textsuperscript{66} identified 53 genes differentially expressed between normal adrenals (n=3) and APAs (n=8). Among them, teratocarcinoma-derived growth factor 1 (TDGF-1) was identified as an upregulated gene in APA. TDGF-1, also known as Cripto-1, belongs to the epidermal growth factor-Cripto-1-cryptic protein family and plays a key role in early vertebrate development and carcinogenesis.\textsuperscript{67,68} TDGF-1 expression is restricted to ZG in normal adrenals, whereas its expression is found in the entire peritumoral adjacent cortex in adrenals with APA. Interestingly, TDGF-1 is localized to nuclei in APAs, whereas it is cytoplasmic in normal adrenal and peritumoral tissue. The possible role of TDGF-1 in APA development was investigated in vitro in the human adrenocortical cell line NCI H295R. In this model, TDGF-1 activates the Akt signaling pathway, mediating increased aldosterone secretion and protecting cells from apoptosis. These results suggest a dual function of TDGF-1 in APA through deregulation of the Akt signaling pathway.\textsuperscript{66}

In their microarray analysis, the same authors also showed that visinin-like 1 (VSNL1) was upregulated in APAs.\textsuperscript{69} VSNL1 belongs to the visinin-like (VSNL) protein subfamily of neuronal calcium sensor proteins.\textsuperscript{70} VSNLs play a role in the transduction of calcium signals and act as modulators of multiple intracellular targets, similar to calmodulin. Interestingly, a recent study identified VSNL1 as a positively regulated target of SF-1, raising the possibility that VSNL1 could be involved in the regulation of adrenal development and steroidogenesis.\textsuperscript{71} In H295R cells, VSNL1 appears to modulate CYP11B2 gene expression and aldosterone secretion and to protect cells from calcium-induced apoptosis.

Interestingly, the expression of VSNL1 was significantly increased in APAs harboring KCNJ5 mutations compared with those without, indicating that VSNL1 may play a key role in the development of KCNJ5 mutant APAs via its antiapoptotic effect in response to calcium cytotoxicity and its role in the regulation of aldosterone production.\textsuperscript{69}

The involvement of the Wnt/β-catenin pathway in the development of adrenocortical carcinomas is now well established.\textsuperscript{72–74} The presence of activating mutations in exons 3 and 5 of the CTNNB1 gene, which encodes β-catenin, has been described in a wide variety of human cancers, including adrenocortical tumors.\textsuperscript{72,75–77} These mutations prevent β-catenin phosphorylation and induce nucleocytoplasmic accumulation of the protein, resulting in constitutive activation of β-catenin target genes. Recently, Berthon et al\textsuperscript{74} showed that mice expressing a constitutively active β-catenin in the adrenal cortex present with adrenal hyperplasia and dysplasia and develop hyperaldosteronism at 10 months, with subsequent evolution toward malignant tumors after 17 months of age. Similarly, mice carrying a mutation in the APC gene, a component of the Wnt/β-catenin degradation complex, develop multiple intestinal tumors but also present with a phenotype of hyperaldosteronism.\textsuperscript{78} These data demonstrate that constitutively active β-catenin is an adrenal oncogene, which can trigger aberrant differentiation of aldosterone-secreting cells and promote malignancy. Recently, we have shown that activation of β-catenin occurs in approximately two thirds of APAs.\textsuperscript{41} However, β-catenin activation status was not associated with any biological or clinical parameters. Moreover, the sequencing of exons 3 and 5 of the CTNNB1 gene in tumor DNA from 41 patients did not show mutations that could account for β-catenin activation. The transcriptome profiles of the Wnt/β-catenin pathway genes were also investigated in 12 APA samples. Interestingly, we observed separate clustering of control adrenals and APA samples supporting changes in the Wnt/β-catenin pathway gene expression between these 2 groups. Moreover, in APAs, those characterized by nonactive β-catenin were distinct from those showing nuclear localization. Surprisingly, APAs with an accumulation of β-catenin in the cytoplasm, which is considered to be the active form, clustered with tumors showing membrane localization, possibly indicating activation of distinct pathways depending on intracellular β-catenin localization.\textsuperscript{41}

Similar to β-catenin, Sonic hedgehog (Shh) plays a key role in adrenocortical development in the mouse. Conditional inactivation of Shh in the adrenal cortex produces severe hypoplasia and histological disorganization.\textsuperscript{79} Moreover, mice null for Shh specifically in SF-1–positive cells show reduced proliferation of capsular cells and a significant reduction of both adrenocortical thickness and adrenal size but no modification of adrenal zonation, indicating that Shh is essential for expansion of the adrenal cortex but not for zonation and differentiation.\textsuperscript{80} In normal human adrenals, Shh expression is restricted to a small number of cells located near the capsule, where stem/precursor cells are assumed to be localized.\textsuperscript{41} Interestingly, we have shown that Shh is expressed in APA; transcriptome analysis of the Shh signaling pathway separated normal adrenals from APA, and APA with homogeneous expression of Shh from those with heter-
Figure. Mechanisms converging toward increased aldosterone production in aldosterone-producing adenoma. The key player in regulating aldosterone biosynthesis is calcium (Ca\textsuperscript{2+}) signaling. Stimulation by angiotensin II (Ang II) or potassium results in depolarization of the zona glomerulosa cell membrane and opening of voltage-dependent Ca\textsuperscript{2+}-channels, leading to an increased intracellular Ca\textsuperscript{2+} concentration. Ang II also signals through the Ang II type 1 receptors (AT1R) to stimulate inositol triphosphate–dependent Ca\textsuperscript{2+} release from the endoplasmic reticulum. Activation of the calcium signaling pathway triggers a phosphorylation cascade, involving calmodulin and calmodulin-dependent kinase IV, leading to the activation of transcription factors (NURR1 and NGF1B, CREB) that bind to the promoter region and positively regulate the transcription of CYP11B2. Gain of function mutations in Kir3.4, as well as inactivation of TASK1 and TASK3 potassium channels, lead to cell membrane depolarization and increased intracellular calcium through the opening of L- and T-type calcium channels. Increased expression of AT1R, as well as ectopic expression of G protein–coupled receptors coupled with G\textsubscript{q/11}, result in activation of phospholipase C (PLC) and hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). DAG increases the activity of protein kinase C (PKC) and protein kinase D (PKD), increasing CREB phosphorylation. The ectopic expression of G protein–coupled receptors coupled to \(\alpha\)- such as the adrenocorticotropic hormone (ACTH) receptor (MC2-R), the luteinizing hormone receptor (LHR), or the serotonin receptor type 4 (5HT4) may result in an increase of intracellular cAMP concentration and, thus, of CREB phosphorylation. Increased expression of teratocarcinoma-derived growth factor 1 (TDGF-1) results in Akt phosphorylation and indirectly in increased aldosterone production. Activation of Wnt/\(\beta\)-catenin signaling may contribute to increased proliferation and aldosterone production. Finally, increased expression of the scavenger receptor B1 promoting increased capture of high-density lipoprotein (HDL) and increased free cholesterol in the cell, together with increased expression of the steroidalogenic acute regulatory protein (SIAR), and steroid biosynthetic enzymes (3\(\beta\)-HSD2) may contribute to aldosterone overproduction.

ogonous expression. Remarkably, we observed a dramatic increase of Shh expression in peritumoral hyperplastic ZG, similar to what we observed previously for CYP11B2 and disabled-2.\textsuperscript{41}

Indeed, adrenals from patients with APA show intense adrenal cortex remodeling and functional ZG hyperplasia, suggesting a potential link with APA formation.\textsuperscript{33} Taken together these results suggest that both APA and the adjacent ZG may have acquired some characteristics of stem/precursor cells or that re-expression of fetal markers from the definitive zone in the adrenal cortex may underlie excessive proliferation and APA formation.\textsuperscript{41} Interestingly, however, KCNJ5 mutations appear to be isolated events in the progression toward APA, because they are not found in the adjacent adrenal cortex of an APA carrying a mutation.\textsuperscript{40} This suggests that KCNJ5 mutations may occur within a proliferating and hyperplastic cortex, leading to growth advantage, clonal expansion, and APA formation in a considerable number of cases. Alternatively, they may represent the primary event leading to APA formation, with adrenal cortex hyperplasia being secondary to reduced vascularization and/or tissue hypoxia. Remarkably, however, KCNJ5 mutations do not induce a unique molecular phenotype of APA, as could have been expected from mutations acting on key signaling path-
ways or master transcriptional regulators. Rather, they may represent one of several possible mechanisms triggering, via increased Ca\textsuperscript{2+} signaling and/or other pathways, increased cell proliferation and aldosterone production (Figure).

Conclusions
Understanding the mechanisms involved in the development of PA is crucial to the development of more powerful diagnostic procedures and possibly innovative therapeutic options, which could benefit \( \leq 10\% \) of hypertensives. Although KCNJ5 mutations play a prominent role in the pathogenesis of both APA and FH3, the genetic determinants and/or the genomic changes occurring in the most common forms of familial and sporadic PA, namely FH2 and BAH, remain to be identified. This will be an exciting challenge for the next decade and may also open new therapeutic perspectives for the management of hypertension in the general population.

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