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Review

Understanding primary aldosteronism: impact of next generation sequencing and expression profiling

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ABSTRACT

Primary aldosteronism (PA) encompasses a broad, heterogeneous group of disorders including both sporadic and familial forms (familial hyperaldosteronism type I, II and III). PA is the most common form of secondary hypertension and associated with a higher rate of cardiovascular complications, compared with essential hypertension. Despite significant progress in the diagnosis and management of PA, until recently the molecular mechanisms leading to inappropriate aldosterone production were largely unknown. The introduction of next-generation sequencing has had a profound impact on the field of human genetics and has given new insight in the molecular determinants that lead to both sporadic and familial forms of PA. Here we review the recent progress toward understanding of the genetic and molecular mechanisms leading to autonomous aldosterone production in PA.

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1. Introduction

Primary aldosteronism (PA) is a heterogeneous group of disorders, characterized by inappropriate aldosterone secretion and concomitant suppression of its main physiological regulator, the renin–angiotensin system. Since its first description by Jerome Conn in 1955 (Conn, 1955) as a secondary form of hypertension associated with hypokalemia and metabolic alkalosis, significant progress has been made in the management of PA patients and in our understanding of the molecular determinants leading to inappropriate aldosterone production. Classically, PA was thought to be a rare and relatively benign form of endocrine hypertension, accounting for less than 2% of all hypertension (Ganguly, 1998). However, over the last 20 years, the widespread use of the aldosterone/plasma renin activity ratio (ARR) as a screening test has led to a 5–15 fold increase in the identification of patients affected by PA (Mulatero et al.,

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2004a), which is now widely recognized as the most common form of secondary hypertension.

Similarly, a wealth of studies have extensively demonstrated the detrimental role of aldosterone in the cardiovascular system (Mulatero et al., 2006), resulting in a higher rate of cardio- and cerebrovascular events, target organ damage, and metabolic syndrome in PA patients compared with essential hypertension with similar blood pressure and risk profiles (Fallo et al., 2006; Milliez et al., 2005; Mulatero et al., 2013a; Savard et al., 2013). In light of these considerations, early detection of PA is of particular importance because it allows for targeted therapy, which has been proven to reverse the excess of organ damage and cardio- and cerebrovascular events (Catena et al., 2008). As recommended by the Endocrine Society Guidelines (Funder et al., 2008), diagnostic work-up for PA is a three-step process that consists of case-finding, confirmation/exclusion and subtype differentiation, based on imaging techniques and selective adrenal vein sampling, to distinguish between unilateral and bilateral forms.

PA is either regarded to be sporadic or hereditary. Approximately 70% of PA patients are affected by bilateral disease, that in the great majority of cases can be attributed to idiopatic hyperaldosteronism (IHA) while the remaining 30% present with a unilateral form are mainly due to aldosterone producing adenomas (APA) (Mulatero et al., 2004a). Rarer subtypes are unilateral adrenal hyperplasia and adrenal carcinoma (Else et al., 2014). Current estimates are that up to 5% of PA are caused by Familial Hyperaldosteronism type I, type II and type III (FH-I to FH-III) (Mulatero et al., 2011, 2013b).

Until recently, the only subtype of PA whose underlying genetic and molecular basis was clearly understood was FH-I (or Glucocorticoid remediable aldosteronism, GRA). GRA is a form of monogenic hypertension transmitted as an autosomal dominant disease. Inappropriate aldosterone production is caused by a hybrid gene, resulting from unequal crossing over between *CYP11B1* and *CYP11B2,* which encode 11beta-hydroxylase and aldosterone synthase, respectively (Lifton et al., 1992). As a result, *CYP11B2* is controlled by the ACTH-responsive promoter of *CYP11B1*. The main clinical features are ACTH-dependent aldosterone secretion, renin suppression and high levels of the hybrid steroids 18-hydroxycortisol and 18-oxocortisol (Mulatero et al., 2004b). Based on these observations, genetic changes in *CYP11B1/B2* were further analyzed in APAs (targeted gene approach). However, despite a moderate increase in hybrid steroid levels and some degree of aldosterone responsiveness to ACTH in APA patients, a chimeric *CYP11B1/B2* gene was not found in sporadic adenomas (Carroll et al., 1996). Similarly, the chimeric *CYP11B1/B2* gene was not present in a large population of IHA patients (Mulatero et al., 1998).

FH-II is a familial form of unknown genetic basis that has been shown to be in linkage with chromosomal region 7p22 in families from different continents (Sukor et al., 2008); however, this linkage has not been demonstrated in other FH-II families (So et al., 2005). FH-III is a severe form of hyperaldosteronism due to mutations in *KCNJ5*, which will be discussed in more detail below in this manuscript.

Until recently, studies of sporadic PA have mainly focused on genetic variants that potentially increase the susceptibility to develop PA or affect the clinical phenotype. Three main polymorphisms of *CYP11B2* have been identified: (i) a c.-344C>T substitution in the promoter region; (ii) an intron 2 gene conversion in which part of intron 2 of *CYP11B2* is substituted with the corresponding region of *CYP11B1* and (iii) a single nucleotide substitution in codon 173 (c.518A>G) leading to a substitution of the arginine with a lysine (Mulatero et al., 2004b). The c.-344C>T polymorphism is located in the putative binding site for the steroidogenic factor 1 (SF1, NR5A1). The biological effect of this variant is unclear: despite SF1 binding being increased in the presence of the c.-344C allele, no effect on *CYP11B2* transcription has been shown by *in vitro* studies (Bassett et al., 2002; Clyne et al., 1997). Interestingly, the c.-344C>T polymorphism has been shown recently to be in tight linkage disequilibrium with another T/C polymorphism at position -1651 (c.-1651T>C) at the binding site of the multifunctional protein DNA (apurinic/apyrimidinic site) lyase called APEX1 (McManus et al., 2012). The substitution affects APEX1 binding and results in different repressor effects on *CYP11B2* transcription; intriguingly, this polymorphism associates also with lower excretion rates of aldosterone metabolites in human subjects (McManus et al., 2012). The gene conversion in intron 2 and the p.Arg173Lys substitution have both been shown to have a strong linkage disequilibrium with the c.-344C>T polymorphism. However, the p.Arg173Lys does not affect aldosterone synthase activity *in vitro* (Portrat-Doyen et al., 1998). Overall, there are several reports that consistently link the *CYP11B2* locus to hypertension and PA not entirely explained by the known polymorphisms (Davies and Kenyon, 2003; Mulatero et al., 2000). Recently, polymorphisms in other genes were found to associate with PA. Polymorphisms in *KCNJ5* and *HSD3β* associate with PA in the Chinese populations (Li et al., 2013; Wu et al., 2013). Finally, polymorphisms in α-adducin (p.Gly460Trp) and bradykinin B2 receptor (p.Cys58Thr) affect blood pressure in PA patients presumably by effects on renal sodium handling (Mulatero et al., 2002).

Over the last few years, the advent of affordable large scale methods of analyses, particularly of gene expression (e.g. transcriptome profiling, cDNA arrays) and gene sequencing (e.g. next generation sequencing, NGS) has dramatically changed the approach to basic, applied and clinical genetic analysis and had a profound impact on genomic variant/mutation discovery. Taking advantage of these next-generation technologies, recent efforts have been directed toward a better understanding of the pathogenic mechanism of the disease. In this review we summarize the impact of these methods on our understanding of the molecular determinants of PA in both sporadic and familial PA.

2. Gene-expression studies

Gene expression profiling is based on hybridization of cDNA, which is generated through reverse transcription from mRNA of the cells or tissue of interest, to short oligomers of DNA. This method was first introduced in the 1990s and has over the last decade become widely available at fairly low cost on different platforms (e.g. Affymetrix, Illumina). In part DNA chip-based technologies have been replaced by direct sequencing of cDNA generated from cellular mRNA (RNAseq). However, it is still a matter of debate whether this NGS based system will show comparable results with the older hybridization based assays. RNAseq has the advantage of defining fusion genes and additional sequence data beyond that found with simple expression data. This method has not been employed for PA samples.

Over the last decade several studies investigated the gene-expression profile of APAs compared with normal adrenals or adjacent adrenal cortex with the aim of identifying transcriptional modulators of aldosterone overproduction. Under physiological conditions, aldosterone is synthesized in adrenal zona glomerulosa cells through the successive action of four different enzymes, cholesterol side-chain cleavage (encoded by *CYP11A1*), type 2 3β-hydroxysteroid dehydrogenase (encoded by *HSD3B2*), 21-hydroxylase (encoded by *CYP21A1*) and finally aldosterone synthase (encoded by *CYP11B2*) (Hattangady et al., 2012). The two main physiological regulators of aldosterone production are angiotensin II (AngII) and serum potassium levels, that exert their effects through increasing cytoplasmic calcium concentration and activating the calcium/calmodulin-dependent protein kinase I/II (CaMKI/II) (Hattangady et al., 2012). This results in the activation of a number of transcription factors such as nuclear receptor related 1 (NURR1)/nerve growth