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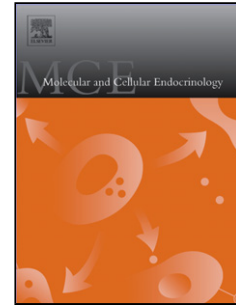
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**Altered corticosteroid biosynthesis in essential hypertension: a digenic phenomenon.**

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**ABSTRACT**

Aldosterone plays an important role in electrolyte and blood pressure homeostasis. Our studies have focused on the role of aldosterone in essential hypertension. We have shown that plasma aldosterone and ARR are heritable characteristics and that aldosterone concentrations in older subjects are inversely correlated with birth weight and positively correlated with blood pressure. Aldosterone levels are also associated with polymorphic variation in the *CYP11B2* gene, which encodes aldosterone synthase, the enzyme responsible for aldosterone production. Interestingly, *CYP11B2* polymorphisms are also associated with less efficient activity of 11 $\beta$ -hydroxylase, encoded by the neighbouring, highly homologous *CYP11B1* gene. We propose that a digenic effect leads to increased aldosterone production, with inefficient 11 $\beta$ -hydroxylation causing a long-term increase in ACTH drive to the adrenal gland and enhanced expression of *CYP11B2*, thereby resulting in chronically-raised aldosterone secretion in response to factors such as angiotensin II and potassium. In susceptible subjects this is likely, over many years, to result in hypertension with relative aldosterone excess.

### **Aldosterone biosynthesis, regulation and action**

Essential hypertension is a common condition affecting one in four adults and is a risk factor for cardio-, cerebro- and renovascular disease (Kearney et al. 2005). Although treatment has improved greatly over the last decade, the basic aetiology is still poorly understood. However, it is known that it is multifactorial, that lifestyle and environmental factors are important and that there are major familial or genetic components. Although the identities of the genes involved remain unknown, the adrenal cortex - and, in particular, the mineralocorticoid hormone, aldosterone - is a major candidate system.

Aldosterone is synthesised from cholesterol in the adrenal zona glomerulosa by a series of enzymatic reactions (Figure 1). The three terminal reactions that convert 11-deoxycorticosterone (DOC) to aldosterone are catalysed by a single enzyme, aldosterone synthase, which is located on the inner mitochondrial membrane and is encoded by the *CYP11B2* gene. The main glucocorticoid, cortisol, is made in the adrenal zona fasciculata from 11-deoxycortisol by the enzyme 11 $\beta$ -hydroxylase, encoded by the *CYP11B1* gene. The *CYP11B1* and *CYP11B2* genes are highly homologous (as are the proteins they encode) and are located in tandem on human chromosome 8 (Chua et al. 1987; Mornet et al. 1989; Wagner et al. 1991). The level of sequence homology between the two genes is lowest in the 5' regulatory region immediately upstream of the coding region, and this is reflected in their differing zonal expression patterns in the adrenal cortex and by marked differences in their regulation.

The principal regulators of aldosterone biosynthesis are the renin-angiotensin system (RAS) and plasma potassium concentration. The octapeptide angiotensin II (AngII) stimulates aldosterone production in response to sodium depletion or a decrease in extracellular fluid volume. Small increases in plasma potassium also stimulate aldosterone production. ACTH, a 39-amino acid peptide synthesised in the pituitary gland as part of the large precursor molecule, pro-opiomelanocorticotrophin (POMC), is the principal regulator of cortisol production but also has an acute stimulatory effect on aldosterone production; our studies suggest it may also play an important role in the long-term regulation of aldosterone synthesis.

Classically, the actions of aldosterone are mediated by mineralocorticoid receptors (MR) located in the cytosol of epithelial cells in the collecting duct of the kidney, the colon and the salivary gland; it also has effects on non-epithelial tissues, including the cardiovascular and the central nervous system (CNS). In epithelial tissues, aldosterone

regulates the reabsorption of  $\text{Na}^+$  – and therefore the transport of water – across the membrane in exchange for  $\text{K}^+$  and  $\text{H}^+$  by altering the expression and activity of ENaC in the apical plasma membrane. A number of aldosterone-induced proteins mediate the alterations in ENaC expression, the best-characterised of which is serine-threonine kinase SGK1 (Stockand 2002; Bhalla et al. 2006).

The MR has an equal affinity for the glucocorticoid, cortisol. As the circulating concentration of cortisol is much higher than that of aldosterone, the majority of these receptors would be expected to be occupied by glucocorticoid. Activation of MR by cortisol in aldosterone target tissue epithelium is prevented by the action of the enzyme  $11\beta$ -hydroxysteroid dehydrogenase type 2 ( $11\beta$ -HSD2) which, in humans, catalyses the conversion of cortisol into cortisone, a steroid that does not cause MR transactivation (Funder et al. 1988; Stewart et al. 1988).

Several studies have detected evidence of aldosterone production, or of the components required for its biosynthesis, in tissues other than the adrenal gland. These include the central nervous system, adipose tissue and the cardiovascular system (Davies & MacKenzie 2003). However, on the basis of the enzymes' and mRNAs' expression rates, this extra-adrenal aldosterone production would be several orders of magnitude lower than that of the adrenal cortex and would be extremely unlikely to contribute to circulating levels of steroid. Nevertheless, the situation of such extra-adrenal production sites in close proximity to corticosteroid receptors means that the physiological significance of such systems cannot be entirely discounted.

### **The role of aldosterone in blood pressure**

There is now substantial evidence that aldosterone plays a key role in developing and/or maintaining hypertension. Initial studies focused on the relatively rare syndrome (1-4% of hypertensives) of Primary Aldosteronism (PA) where excess aldosterone production results in hypertension, hypokalaemia and a metabolic alkalosis. Aldosterone production is independent of regulation by renin (and AngII), so cannot be suppressed with sodium or volume loading. In most cases this was attributable to a unilateral aldosterone-producing adenoma (APA).

However, the extensive use of the aldosterone to renin ratio (ARR) as an index of abnormal aldosterone production together with the introduction of screening programmes of hypertensive patients has led to a revised estimate that 8-15% of hypertensive patients have a

raised ARR (Mulatero et al. 2004; Rossi et al. 2006; Young 2007), with even higher frequencies (~22%) detected in specially-selected populations such as patients with resistant hypertension (Calhoun et al. 2002). One of the most recent studies from Italy (PAPY) estimates that the frequency of subjects with a high ARR in an unselected hypertensive cohort is 11.2% (Rossi et al. 2006); this is likely to be accurate given the careful design of the study. Although a high ARR indicates dissociation of aldosterone from normal regulation by renin and AngII, it does not necessarily imply the presence of an APA. Indeed, less than 50% of the PAPY subjects with a raised ARR, (~4% of the total population of hypertensives), were found to have a solitary APA. A raised ARR in the remaining patients is due to excess aldosterone production from both adrenal glands i.e. bilateral adrenal hyperplasia (BAH). Whether this is defined as PA is largely a matter of semantics. Compared to patients with APA, levels of renin and AngII are markedly less suppressed and aldosterone exhibits an enhanced response to AngII and potassium. If the ARR is used to diagnose PA, then patients with bilateral adrenal hyperplasia will be classified as having the disorder. However, as the aldosterone responses to AngII (and potassium) are steeper than normal, it may be argued that they have inappropriately high (in relation to concurrent renin concentration), rather than autonomous, secretion of the hormone.

While these studies have focused on hypertensive subjects with excess aldosterone, it is now clear that variation in aldosterone levels within the normal range has a marked effect on blood pressure. In the Framingham non-hypertensive offspring cohort, subjects with aldosterone values in the top quartile of the range showed a greater increase in blood pressure over a five-year period than subjects with aldosterone measurements in the bottom quartile. In addition, a greater number of subjects with aldosterone measurements in the top quartile developed overt hypertension (Vasan et al. 2004). Our own studies confirm these findings. Aldosterone levels in a small cohort of elderly subjects correlated positively with systolic blood pressure. Blood pressure in subjects in the top tertile of aldosterone levels was approximately 10mmHg higher than those in the bottom tertile and this was not confounded by treatment (Reynolds et al. 2007).

### **Regulation of aldosterone production by genetic and environmental factors**

It is clear that there is significant basal variation in aldosterone levels and that this is likely to be determined by the complex interaction between a wide range of factors, be they clinical, environmental (e.g. sodium intake), genetic or a combination of these, such as early life programming.

Aldosterone levels vary with gender, age, ethnicity and BMI (James et al. 1976; Weidmann et al. 1978; Luft et al. 1979; Tsunoda et al. 1986; Goodfriend et al. 1998; Olivieri et al. 2004). Anti-hypertensive treatments have well-recognised effects on the aldosterone levels and the derived ARR. In the Framingham offspring cohort, aldosterone levels were higher in subjects using diuretics (Kathiresan et al. 2005); the ARR was positively associated with beta-blockers and HRT and negatively associated with diuretics and ACE inhibitors (Newton-Cheh et al. 2007).

Sodium intake has an important effect on aldosterone regulation (Oelkers et al. 1974) mainly by altering sensitivity of aldosterone synthesis to its principal stimulus, AngII. This was confirmed in the Framingham offspring study, where urinary sodium was the strongest correlate of serum aldosterone ( $R^2$  10%) (Vasan et al. 2004).

The effect of *in utero* programming has not been examined in great detail but our own studies show that there is an inverse correlation between recorded birthweight and aldosterone concentration in adulthood (Reynolds et al. 2007). The exact mechanism of this programming effect is unclear, but it is reminiscent of studies by Barker who showed that low birthweight was associated with increased incidence of cardiovascular disease in adulthood. Crucially, in Barker's studies, cortisol levels tended to be higher in the low birthweight group (Phillips et al. 2000).

### **Genetic determination of aldosterone levels**

When attempting to establish whether there is a genetic influence over a phenotype, it is useful first to determine whether there is any evidence of familial clustering or heritability. The Framingham population is a large normotensive cohort of 3326 subjects in which heritability of multivariate adjusted logARR was 0.4 ( $p < 10^{-4}$ ). Studying normotensive monozygotic and dizygotic twins gave no evidence of a genetic influence on plasma aldosterone concentration; urinary excretion of aldosterone, on the other hand, was clearly heritable in another study (Inglis et al. 1999). In contrast, plasma aldosterone in the Framingham offspring population was only modestly heritable ( $h^2=0.1$ ,  $p < 0.04$ ) (Kathiresan et al. 2005); this was confirmed in a study of 43 hypertensive sibling pairs ( $h^2=0.19$ ) (Kotchen et al. 2000). It was clear from these studies that short-term environmental changes in dietary salt, potassium and posture could obscure the true heritability of aldosterone, as determined by single plasma aldosterone samples. Measurement of the 24-hour urinary excretion of tetrahydroaldosterone (THaldo), the principal metabolite of aldosterone, is not subject to these intra-diem changes. Indeed, the high genetic heritability of urinary



aldosterone excretion reported by Inglis *et al* (see above) has now been confirmed in a large collection of nuclear families ( $h^2=0.52$ ,  $p<10^{-6}$ ) (Imrie *et al.* 2006).

So, the belief that there is a modest genetic influence on aldosterone status, as assessed by its urinary excretion and plasma levels, is now supported by considerable evidence. The gene encoding the aldosterone synthase enzyme, *CYP11B2*, is the most obvious candidate gene for investigation and rare human monogenic disorders, together with rodent models of hypertension, show that variation at this locus does influence blood pressure and aldosterone synthesis.

The Dahl rat demonstrates how an environmental factor – in this case, salt – and genetic factors interact to result in a hypertensive phenotype. The importance of the *CYP11B1* and *CYP11B2* two-gene locus in altering corticosteroid biosynthesis and in the pathogenesis of high blood pressure is also apparent. The two Dahl rat strains were originally selected according to the blood pressure they achieved on high salt diets; the salt-sensitive (SS) strain became severely hypertensive upon salt loading while a less dramatic effect was observed in the salt-resistant (SR) strain. The principal rodent glucocorticoid is corticosterone, synthesised from DOC by  $11\beta$ -hydroxylase, an enzyme also capable of catalysing corticosteroid 18-hydroxylation of deoxycorticosterone to form 18-hydroxydeoxycorticosterone (18-OH-DOC), a weak mineralocorticoid. In comparison to SR animals, SS animals synthesise proportionally greater levels of 18-OH-DOC relative to corticosterone (Rapp & Dahl 1972) and this could be attributed to one, or a combination, of the five mutations in exons 2, 6, 7 and 8 of the SR strains' *CYP11B1* gene (R127C, V351A, V381L, I384L and V443M), which associate with altered  $11\beta$ -hydroxylase activity and blood pressure (Cicila *et al.* 1993; Matsukawa *et al.* 1993; Nonaka *et al.* 1998; Cicila *et al.* 2001). In contrast, attributing the salt-sensitive hypertensive phenotype to genetic changes in *CYP11B2* is less easy. While two nonsynonymous mutations (Glu136Asp and Glu251Arg) have been identified at this locus in the Dahl SR rat (Cover *et al.* 1995), these result paradoxically in increased aldosterone synthase activity.

### **The *CYP11B1/CYP11B2* locus in essential hypertension**

The *CYP11B1* and *CYP11B2* genes lie approximately 40kb apart on human chromosome 8 and have been identified through studies of animal models and rare human monogenic syndromes as obvious candidate genes in essential hypertension (Mornet *et al.* 1989). The genes each consist of 9 exons and are 95% identical within their coding regions,

falling to 90% identity in the introns. Numerous polymorphisms have been identified across the *CYP11B1/CYP11B2* locus and there is relatively tight linkage disequilibrium (LD) across it, resulting in a limited number of common haplotypes (Figure 2). Among the various polymorphisms identified throughout the coding and regulatory regions of the genes, most attention has been paid to a single nucleotide polymorphism (SNP) at the -344 position of the *CYP11B2* gene, which is found in either of two forms (T and C) with roughly equal frequency in the general population. Although much of the initial interest in this polymorphism resulted from its location within a putative Ad4 *cis*-element, capable of binding steroidogenic factor 1 (SF-1) (White & Slutsker 1995), *in vitro* studies could not demonstrate any significant differences in gene transcription resulting from the two alternate forms (Bassett et al. 2002). A gene conversion, whereby part of intron 2 of the *CYP11B2* gene is replaced with the corresponding region of *CYP11B1*, has also been studied in detail (White & Slutsker 1995). It is not yet known whether this region contains sequences that might regulate gene transcription.

**Association with blood pressure:** Several studies, including our own, have concluded that the -344 T polymorphism and the intron 2 conversion (as opposed to the 'wild type') are more frequent in patients with essential hypertension than their alternative forms (Brand et al. 1998; Davies et al. 1999; Paillard et al. 1999; Komiya et al. 2000). We also reported that hypertensive patients with an elevated ARR (>750) are more likely to possess the -344T allele than subjects with a normal ratio (Lim et al. 2002). However, other groups have been unable to identify such an association between -344T and hypertension, while others have reported that it is the C allele that associates with increased blood pressure (Pojoga et al. 1998; Tsujita et al. 2001). Despite these contradictory findings, a recent meta-analysis designed to address this controversy did conclude that the -344T allele is indeed associated with a higher, though modest, risk of hypertension, but that it has minimal influence on aldosterone excretion (Sookoian et al. 2007). It is worth noting, however, that all of the studies published to date, including those within the meta-analysis, analysed relatively few subjects. Where the effects of genes exerting relatively minor effects on complex traits, such as obesity, have been identified (generally conferring odds ratios of approximately 1.2), significantly larger case/control groups are necessary (Frayling et al. 2007). Therefore, in order to implicate the *CYP11B1/CYP11B2* locus in essential hypertension, we require a large, appropriately powered case/control analysis using suitably-defined tag SNPs that can identify common haplotypes, together with independent confirmation in a separate population.

**Association with aldosterone synthesis:** If an association does indeed exist between this locus and hypertension, the mechanism that links the genotype to aldosterone synthesis must be considered. Although the studies mentioned above detected no altered regulation of *CYP11B2* expression, several others have demonstrated associations between aldosterone production and allelic variants at the *CYP11B2* locus. This includes the -344 SNP, which we now know to be in tight LD with the intron 2 conversion and another polymorphism, K173R (Fardella et al. 1996a; White & Slutsker 1995; Fardella et al. 1996b). Since we initially reported that the -344T allele was associated with increased THaldo excretion rate (Davies et al. 1999), subsequent investigations have concluded that plasma levels of aldosterone are increased in -344T subjects (Paillard et al. 1999). The K173R, found in the gene's coding region, was first described in a Chilean population where the R variant was found to be more common in hypertensive subjects with suppressed renin, although the polymorphism did not alter enzymatic activity *in vitro* (Fardella et al. 1996b). More recent studies assessing levels of *CYP11B2* mRNA in both normal adrenal tissue and aldosterone-producing adenomas suggest that the -344T/K173 haplotype is associated with higher levels of gene expression than the alternate -344C/R173 haplotype (Tanahashi et al. 2005).

Therefore, in spite of this lack of concordance amongst the data, it does appear that the -344T, the intron 2 conversion and the K173 polymorphisms of *CYP11B2* all associate with increased aldosterone synthesis. However, a more complex picture has emerged in the wake of our studies into association between urinary THaldo excretion and a number of other variants across the *CYP11B2* and also the *CYP11B1* genes (Imrie et al. 2006). In a large family-based study derived from hypertensive probands, we demonstrated a relatively weak but statistically significant association between THaldo excretion and the polymorphisms found at the -344 and intron 2 positions ( $R^2$  1.1% and 1.4%, respectively). Surprisingly, however, it was a SNP within the *CYP11B1* (11 $\beta$ -hydroxylase) gene that showed the strongest association with variations in THaldo excretion (Keavney et al. 2005). Despite being a highly heritable characteristic, this study showed that polymorphic variation in *CYP11B2* and *CYP11B1* only accounts for up to 10% of the observed variability in THaldo excretion. Furthermore, a subsequent study could not identify any association between plasma aldosterone levels and polymorphic variation in the *CYP11B2* and mineralocorticoid receptor (MR) genes (Kathiresan et al. 2005). Therefore, it is clear that other genetic factors, such as those encoding other components of the Renin Angiotensin System (RAS), must

contribute to the observed variability in aldosterone status. This has yet to be studied in detail but nevertheless leads us to consider how variations in the regulation of aldosterone excretion might interact with changes in the secretion of other corticosteroids in instances of cardiovascular disease.

### **Association of 11 $\beta$ -hydroxylation efficiency with hypertension**

We have already discussed the fact that altered aldosterone metabolism has an impact on blood pressure in man. However, there is also evidence of changes in the metabolism of other corticosteroids. The ratios of plasma steroid concentration which are generally accepted as indices of impaired 11 $\beta$ -hydroxylase efficiency – namely 11-deoxycortisol (S) to cortisol (F), DOC to corticosterone (B), or the ratios of their respective urinary metabolites – are more frequently raised in hypertensive subjects than in control normotensives. This phenomenon was first discovered by means of ACTH stimulation (de Simone et al. 1985; Hautanena et al. 1998) but has since been consistently demonstrated in unstimulated subjects (Soro et al. 1995; Ganapathipillai et al. 2005). This phenomenon is distinct from that of classical 11 $\beta$ -hydroxylase deficiency; there is no evidence of cortisol (or corticosterone) deficiency and the increased ratio results instead from higher levels of steroid precursor (i.e. S or DOC). Thus, in hypertensive subjects, it appears that the system of corticosteroidogenic enzymes is, on average, physiologically adequate but, in comparison to normal controls, relatively inefficient, requiring higher concentrations of substrate to achieve a normal level of production. Such increased levels of substrate are likely to be achieved at the cost of marginally raised ACTH. Although a higher DOC to corticosterone (and deoxycortisol to cortisol) ratio provides a useful marker of this effect, absolute levels of DOC, a very weak mineralocorticoid, are unlikely to be the cause of any additional MR activation in the presence of normal circulating levels of aldosterone.

Heritability studies, such as those described above in relation to aldosterone, are useful in identifying a possible genetic component to a phenotype. Twin and other family studies also show that the S:F ratio in plasma and urine are strongly heritable traits. Moreover, *CYP11B2* and *CYP11B1* – logical candidate genes for essential hypertension – are also implicated in the phenotype of apparent altered 11 $\beta$ -hydroxylation efficiency. We previously reported that the -344T allele of *CYP11B2*, implicated in hypertension with a raised ARR, is also associated with a higher S:F ratio in normal subjects (Davies et al. 2001; Lim et al. 2002). A similar finding, relating to variation in *CYP11B1*, has also been published

(Ganapathipillai et al. 2005) and we have corroborated this in a large population of hypertensive patients and also in a separate family-based population study (Keavney et al. 2005).

There are, therefore, convincing data to support the assertion that a raised ratio of 11-deoxysteroid to product (S:F or DOC:B) is accounted for by sequence variation at the *CYP11B1* gene, and our recent studies provide a plausible explanation for this. Briefly, two SNPs in the 5' untranslated region of *CYP11B1* (-1889G/T and -1859A/G) are associated with altered *in vitro* transcription of a reporter gene construct in response to ACTH (Barr et al. 2007) (see Figure 3). The -1889T and -1859G forms, which result in reduced *in vitro* transcription, associate with higher S:F ratios *in vivo*; this is consistent with impaired 11 $\beta$ -hydroxylase efficiency. Furthermore, these SNPs are in close LD with the -344T allele of *CYP11B2*. These data have allowed us to define a specific, single haplotype which is associated with an altered S:F ratio, increased aldosterone production and higher risk of hypertension. This raises the further question of whether the parallel blood pressure-associated changes in aldosterone and cortisol synthesis are independent – and therefore simply coincidental – or whether they are causally linked. While, on the face of it, either case could be true, we believe that there is compelling evidence to suggest that genetically-determined variation in 11 $\beta$ -hydroxylase efficiency is the primary intermediate phenotype and that changes in aldosterone level and blood pressure are direct consequences of this, mediated by very small but lifelong changes in ACTH drive to the adrenal cortex.

Could such a small rise in ACTH drive, extended over a lifetime, really be responsible for the observed rises in aldosterone concentration and, furthermore, sufficiently alter the aldosterone/renin balance to result in increased ARR? We believe so. The synthesis of aldosterone depends, like any other corticosteroid, on the mobilisation of cholesterol for initial side-chain cleavage. The principal agent, the Steroidogenic Acute Regulatory protein (StAR), is activated during mineralocorticoid synthesis in the rat (Cherradi et al. 1998) and ACTH is a powerful activator of StAR. In spite of this, it is widely believed that ACTH is not an important driver of aldosterone secretion. However, this belief is largely founded on studies in which ACTH administration has been shown to initially stimulate aldosterone production before returning to basal levels and, eventually, suppressing it. These studies involved the administration of high levels of ACTH over short time periods (Tucci et al. 1967; Fuchs-Hammoser et al. 1980; McDougall et al. 1980; Oelkers 1985). High sodium status tends to attenuate aldosterone's response to all its stimulatory regulators, and high

doses of ACTH promote sodium retention which will then attenuate responsiveness (Connell et al. 1987). Preventing sodium accumulation by ACTH infusion into sodium-restricted subjects results in prolonged stimulation of aldosterone production (Tucci et al. 1967). A sustained increase in THAldo excretion rate over four days of ACTH infusion has been reported (Pratt et al. 1976), while more than one study has reported that levels of plasma aldosterone are more sensitive to ACTH stimulation than either cortisol or DHEA (Kem et al. 1975; Daidoh et al. 1995). Biglieri found aldosterone to be within the normal range – but not increased – in Cushing’s syndrome (Biglieri et al. 1963).

The idea that ACTH is an important component in the routine control of aldosterone secretion is supported by several studies that show a strong similarity in the circadian and pulsatile pattern of cortisol and aldosterone concentrations (Katz et al. 1975; Schambelan et al. 1976; James et al. 1976; Richards et al. 1986). On a related note, hypophysectomised rats progressively lose their ability to increase aldosterone production in response to sodium depletion, although this cannot be restored by ACTH administration; other pituitary factors, such as  $\gamma$ -MSH, might be involved (Griffing et al. 1985). Recent evidence shows that the POMC knockout mouse has abnormal adrenocortical morphology and reduced levels of aldosterone, further suggesting that ACTH is required for normal aldosterone secretion (Coll et al. 2004). In addition, human subjects with mutations in genes encoding the adrenal ACTH receptor or its essential accessory protein have altered regulation of aldosterone (Lin et al. 2007).

Recently, we showed, in a large population survey, a close correlation between urinary cortisol metabolites, ACTH-dependent adrenocortical androgen metabolites and THAldo excretion rates (Freel et al. 2007). This correlation could be demonstrated only in subjects homozygous for the -344T *CYP11B2* polymorphism, consistent with the hypothesis that, in subjects with a relative reduction in 11 $\beta$ -hydroxylase efficiency, a minor but chronic increase in ACTH drive to the adrenal gland results in a higher dependence of aldosterone secretion on POMC-derived peptides. In addition, we showed (Freel et al. 2008) that subjects with the -344T allele in *CYP11B2* had ACTH levels that were higher in the early morning than subjects with the contrasting allele. As this same variant (-344T) is the one apparently associated with less efficient 11 $\beta$ -hydroxylation, we have corroborative evidence that there is a compensatory increase in activity of the HPA axis.

On the basis of this evidence, it seems reasonable to suggest that ACTH plays a significant role in the control of aldosterone excretion. Therefore, genetic variations in

*CYP11B1* that result in modified gene transcription and/or changes in enzyme activity could result in increased compensatory levels of ACTH and, therefore, aldosterone. The mechanism by which ACTH stimulates aldosterone may be two-fold. Firstly, it may act as a direct stimulus of aldosterone excretion or, alternatively, it may promote hypertrophy and hyperplasia of the adrenal zona glomerulosa, thereby increasing sensitivity to AngII and potassium. If the latter possibility is true, bilateral adrenal hyperplasia with aldosterone excess, the aetiology of which is currently unknown. This theory offers a testable hypothesis for investigating the origins of hypertension with relative aldosterone excess.

### Conclusions

It is clear from studies by ourselves and others that polymorphic variation at the *CYP11B2* gene is associated with increased risk of hypertension. In the course of these studies, it has also emerged that variations in the neighbouring *CYP11B1* gene also appear to contribute to dysregulation of aldosterone control, leading us to propose that the changes in corticosteroid biosynthesis that are observed in essential hypertension result from a digenic effect in which the production of aldosterone synthase and 11 $\beta$ -hydroxylase both play a role. Here we have recounted the evidence that led us to this conclusion and have laid out the theory which, in the course of our future studies, we aim to test.

The RALES and EPHEBUS studies demonstrated the myocardial protective effect of MR blockade which significantly reduced overall mortality and cardiovascular mortality (Pitt et al. 1999; Pitt et al. 2003). This suggests that blockade of MR or, alternatively, the inhibition of aldosterone synthesis, could become major therapeutic targets in the future and illustrates the necessity of a thorough understanding of normal control of aldosterone synthesis and the aberrations of this which occur in essential hypertension. The digenic influence described here, leading to important inputs from agonists other than AngII, is an important contribution to this understanding.

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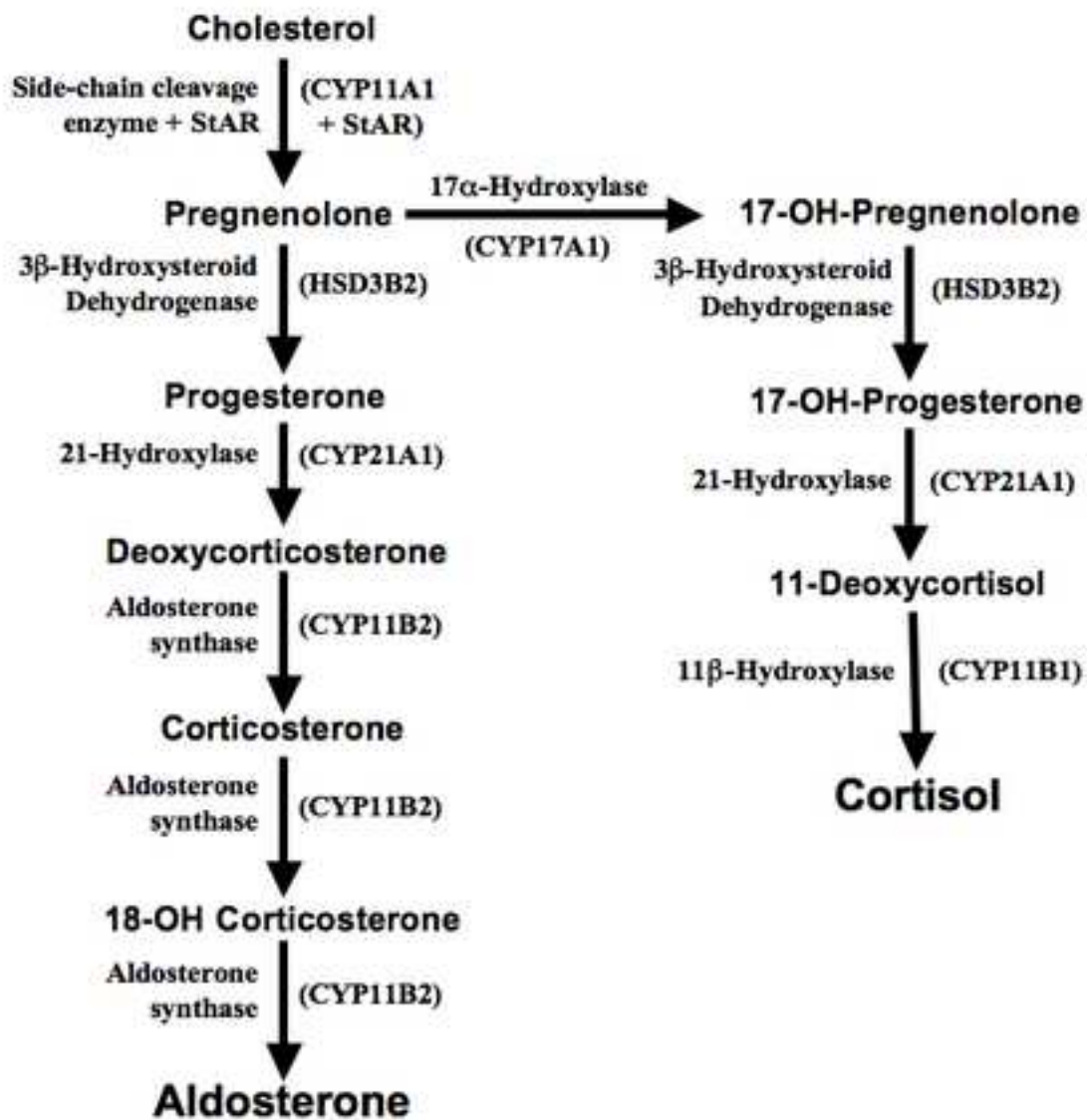
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**FIGURE LEGENDS**

Figure 1 Pathway showing the biosynthetic conversion of cholesterol to aldosterone and cortisol. The names of the biosynthetic enzymes are shown together with the names of the relevant genes in brackets.

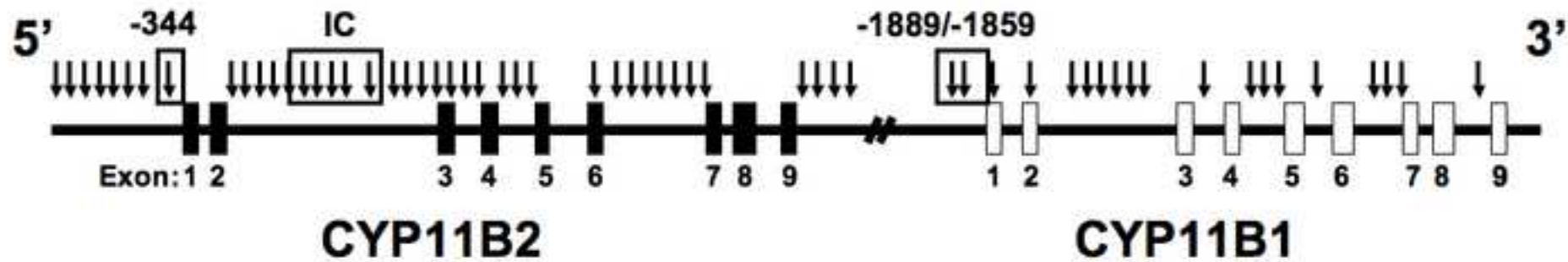
Figure 2 The structure of the human *CYP11B1* and *CYP11B2* genes, indicating common polymorphisms in linkage disequilibrium with the -344 polymorphism across the locus. The boxed arrows indicate the -344 polymorphism itself, the polymorphisms associated with the intron 2 conversion in *CYP11B2* (IC) and the -1889/-1859 polymorphisms upstream of *CYP11B1*.

Figure 3 Luciferase reporter gene activity of *CYP11B1* gene promoter constructs in the Y1 adrenal cell line. Polymorphisms at the -1889 and -1859 positions were tested for their response to 1 $\mu$ M ACTH. Luciferase activity levels are relative to an unstimulated control (not shown). The T/G polymorphisms at the -1889/-1859 positions, respectively, resulted in approximately 35% lower expression compared to the opposite G/A genotype. (Adapted from Barr *et al.*, 2007.)





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