Dissecting hypertension: the role of the 'new genetics'

ABSTRACT—The tools of molecular genetics have recently been applied to hypertension, a common multifactorial disorder, with some success. Glucocorticoid-suppressible hyperaldosteronism, an inherited form of human hypertension due to the dominant inheritance of a chimaeric steroid 11β-hydroxylase/aldosterone synthase gene, has given an insight into the possible genetic factors involved in essential hypertension. Study of the aldosterone synthase and steroid 11ß-hydroxylase genes has shown the presence of polymorphisms in both of these genes in human subjects; further studies may demonstrate genetic mutations with pathophysiological effects in patients with essential hypertension.

Since the debates of Pickering and Platt in the early years of hypertension research it has been recognised that there is a genetic component to the development of hypertension. This genetic contribution accounts for about 30% of blood pressure variability and is likely to be polygenic in nature, perhaps due to 4–6 genes. However, which genes are important is not clear and it is difficult to dissociate the confounding effects of environmental influences from the suspected genetic loci.

Within the past few years, evidence of a role for a small number of 'candidate' genes in hypertension and ischaemic heart disease has been reported. Candidate genes are genes for enzymes or peptides of importance in physiological processes relevant to the condition being studied. Examples include the genes for angiotensin I converting enzyme (ACE) and angiotensinogen (AGT), key components of the renin-angiotensin system. Such studies have tried to determine first whether a polymorphism exists in or near the region of the candidate gene and second whether a particular allele of the polymorphism is linked to the disease phenotype; that is, does one allele of the suspect polymorphism occur with greater frequency in affected individuals than in appropriate controls? Two such polymorphisms have been studied in some detail.

One is the ACE insertion/deletion polymorphism (ACE I/D) for which there is no evidence of linkage to essential hypertension [1] but which is linked to a particular genotype, the DD genotype which develops left ventricular hypertrophy in hypertension and myocardial infarction [2,3]. The other polymorphism involves the AGT gene which shows linkage to both hypertension and preeclampsia [4–7]. Unfortunately these polymorphisms of candidate genes of the renin-angiotensin system are not associated with a clear physiological abnormality sufficient to support a genetic link on the basis of their known physiological actions.

Another approach to the study of hypertension is to study subgroups of hypertensive patients who consistently display a well defined physiological abnormality in association with hypertension, a so-called intermediate phenotype which may have a genetic basis. One such subgroup of patients is individuals with glucocorticoid-suppressible-hyperaldosteronism (GSH). This is an uncommon form of hereditary hypertension characterised by an autosomal dominant mode of inheritance, high plasma aldosterone concentrations associated with suppression of plasma renin, reversed by the administration of glucocorticoids (dexamethasone 0.5 mg qds for four weeks) [8,9].

Adrenal cortex physiology and molecular biology

The normal human adrenal cortex secretes two major steroid hormone products—aldosterone from the outer zona glomerulosa and cortisol from the inner zona fasciculata. In addition to the anatomical separation of hormone synthesis, the synthesis and secretion of these two hormones are independently regulated; cortisol synthesis is stimulated by adrenocorticotrophin (ACTH) via the hypothalamic-pituitary-adrenal axis, and aldosterone predominantly via angiotensin II (Ang II) formed from angiotensinogen via renin and ACE (Fig 1). Both cortisol and aldosterone are formed from a common precursor, cholesterol, mainly by a series of hydroxylation reactions. The zona glomerulosa lacks the enzyme 17α-hydroxylase, which is active in the zona fasciculata, and as a result makes only 17–deoxysteroids.

Of particular interest to the discussion of GSH are the 'late reactions' in corticosteroidogenesis, ie the conversion of 11-deoxycortisol into cortisol and the conversion of 11-deoxycorticosterone into aldosterone (Fig 2). The final step in the formation of cortisol is carried out by the enzyme 11β-hydroxylase, a
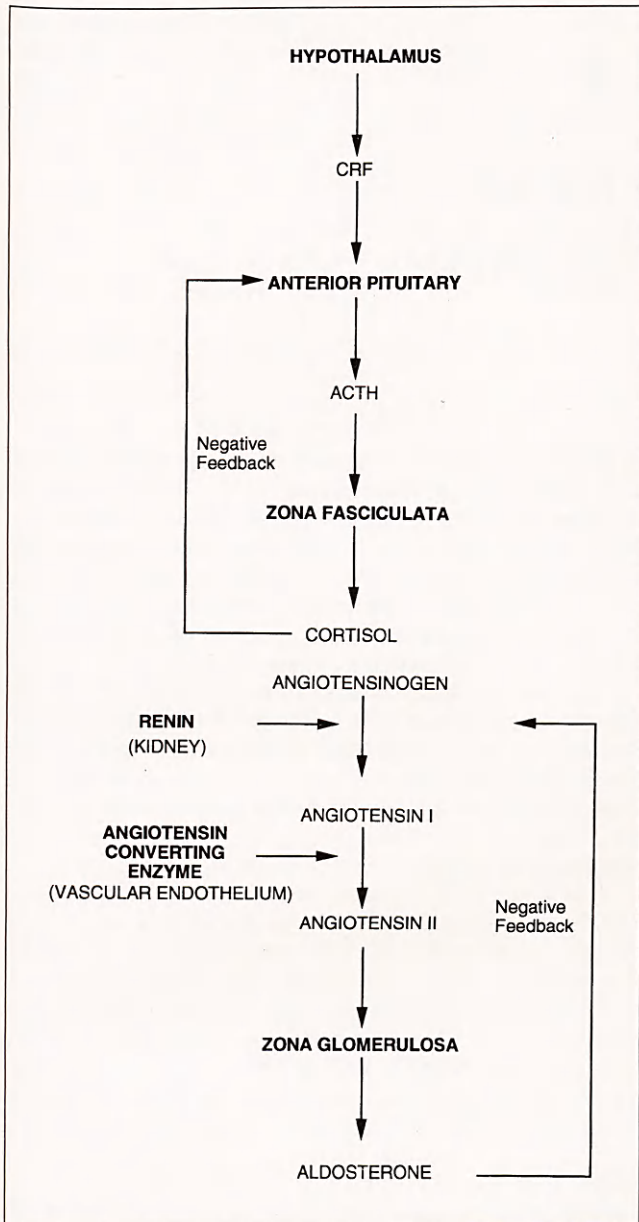
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Fig 1. *Major factors in the control of aldosterone and cortisol secretion in man.*

Fig 2. *The 'late reactions' in the synthesis of aldosterone and cortisol.* In patients with GSH two hybrid steroids are formed, 18-hydroxycortisol and 18-oxocortisol, which are analogous to the zona glomerulosa specific steroids 18-hydroxycorticosterone and aldosterone.

cytochrome P450 enzyme encoded by the gene CYP11B1 found on chromosome 8q22 [10]. This enzyme is highly active in the fasciculata, and *in vivo* can convert 11-deoxycortisol into cortisol and 11-deoxycorticosterone into corticosterone. The gene for this enzyme has an ACTH-responsive element in its promoter region, and cDNA constructs expressed in COS cells show ACTH-inducible 11β-hydroxylase activity. In addition, mutations of this gene in man result in clinical 11β-hydroxylase deficiency. Such mutations may be either point mutations predominantly in exons 6–8 of CYP11B1 and result in amino acid changes in the gene product which diminish the activity of the
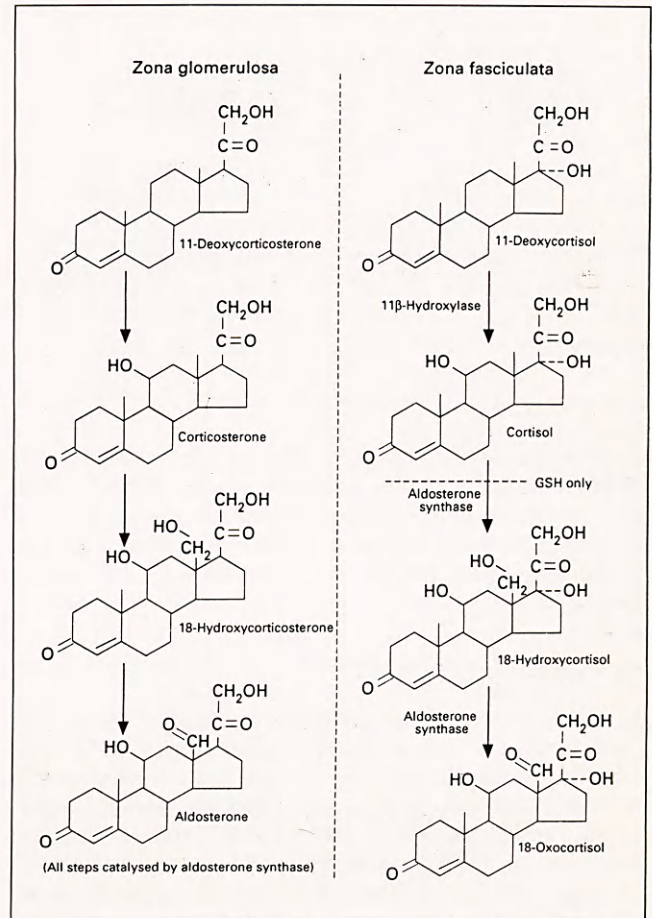
encoded enzyme—missense mutations [10–12]—or they may be frame-shift mutations where, in the case of 11β-hydroxylase deficiency, two nucleotides have been inserted into the normal gene and as a result the normal nucleotide sequence has been altered, giving rise to a truncated functionally inactive protein product [13].

In the glomerulosa, the final three hydroxylation steps in the synthesis of aldosterone are catalysed by one enzyme, aldosterone synthase [14]. It is also a cytochrome P450 enzyme and is encoded by a gene, CYP11B2, situated approximately 40 kilobases upstream in tandem with CYP11B1 [15,16]. These two genes share a very high degree of nucleotide similarity or homology: they are 95% similar in their exonic and amino acid sequences and 90% homologous in their intronic sequences [10]. Studies similar to those involving CYP11B1 have shown that the expression of aldosterone synthase is regulated by Ang II and that mutations in CYP11B2 are responsible for clinical
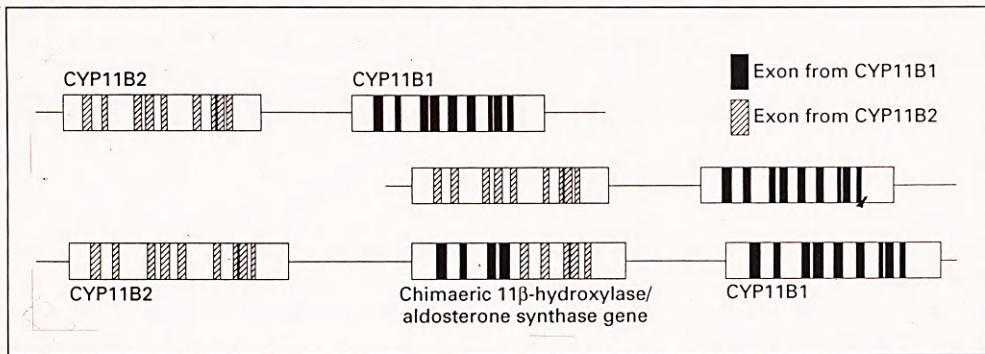
Fig 3. *Schematic representation of the events resulting in the formation of a chimaeric 11β-hydroxylase/1aldosterone synthase gene.*

aldosterone deficiency (corticomethyloxidase type II deficiency) [14,17]. For example, the mutations giving rise to corticomethyloxidase type II deficiency are of a similar type to those causing the majority of cases of 11β-hydroxylase deficiency, ie missense mutations in CYP11B2.

Pathophysiology of GSH

In GSH, aldosterone synthesis is ACTH-dependent. Aldosterone secretion is hypersensitive to ACTH infusion and is suppressed by dexamethasone which suppresses ACTH secretion; it follows the same nycthemeral rhythm as ACTH and cortisol. There is elevated secretion of the hybrid steroids 18-hydroxycortisol and 18-oxocortisol, 17α-hydroxylated analogues of 18-hydroxycorticosterone and aldosterone [18,19] (Fig 2). These are synthesised from cortisol, a precursor which does not occur in the zona glomerulosa. This is strong evidence of ectopic expression of aldosterone synthase in the zona fasciculata. These two observations suggest that in GSH aldosterone synthase is expressed throughout the adrenal cortex and its activity is regulated by ACTH, perhaps as the result of

a genetic mutation involving the genes CYP11B1 and B2. A mutation involving these genes is present in all affected subjects [9,15,17,20]; they inherit a chimaeric gene formed from the 5' portion of CYP11B1 which includes its ACTH-responsive element, and a 3' portion of CYP11B2 which confers aldosterone synthase activity on the resultant gene product. Such a chimaeric gene is thought to arise from a historical crossover event during meiosis; made possible by the high homology between CYP11B1 and B2 (Fig 3). A similar mechanism may be involved in the production of inactive genes in certain forms of congenital adrenal hyperplasia due to 21-hydroxylase deficiency [21].

Diagnosis of GSH

Individuals with GSH can now be detected using a simple genetic test based on the technique of Southern blotting. DNA is prepared from peripheral leucocytes of individuals suspected of having GSH and digested with the restriction enzyme BamHI. This enzyme cuts DNA at specific sites throughout the entire genome determined by a specific series of nucleotides. The DNA is then fractionated by size on

Fig 4. *A typical result from probing BamHI-digested DNA from individuals with and without GSH.* The upper and lower bands correspond to signals from CYP11B2 and CYP11B1 respectively. The middle band, found only in patients with GSH, arises from the chimaeric gene.
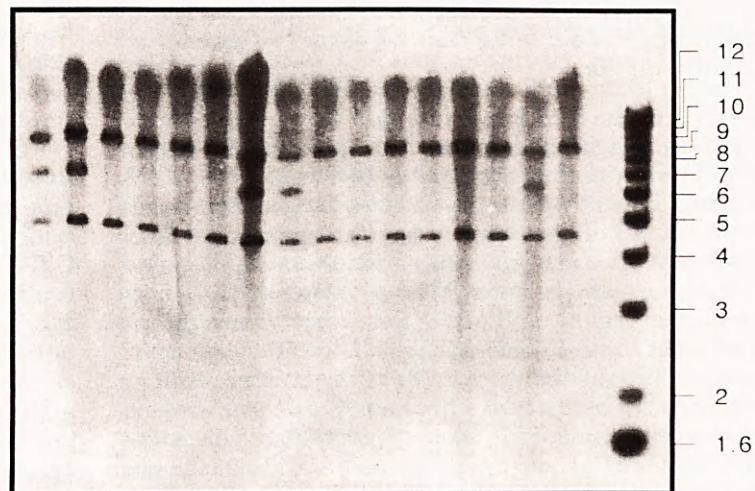
an agarose gel and transferred to a nylon membrane which is exposed to a radiolabelled probe prepared from exons 2–5 of CYP11B1. In normal individuals, this technique gives rise to two hybridising species, a 4.2 kilobase band corresponding to CYP11B1 and an 8.3 kilobase band corresponding to CYP11B2. In individuals with GSH, a third band of 6.3 kilobases corresponding to the abnormal chimaeric CYP11B1/B2 gene is found in addition to the other two bands (Fig 4), a finding 100% diagnostic of GSH [9,22,23].

Genotype versus phenotype in GSH

The site of crossovers occurs in the region of introns 2–5 of the chimaeric gene. Such chimaeric genes encode enzyme products with aldosterone synthase activity but with different amino acid sequences. Their predicted amino acid sequence may differ from others by only one amino acid and the potential for variation between kindreds in aldosterone synthase activity, and thus plasma aldosterone concentration and blood pressure, may be limited (A Jamieson, unpublished data). However, observations from our own group suggest that blood pressure is a relatively poor predictor of the presence of GSH in an individual, and within the same kindred wide variations in blood pressure level may occur at a given age. Why such variations should occur is not clear but environmental influences such as high sodium intake, which may enhance the level of blood pressure in an individual for a given plasma aldosterone concentration, could be important factors in the alteration of the phenotypic expression of the chimaeric gene.

Relationship of GSH to essential hypertension

Can we relate the findings of abnormalities in the genes for steroid hydroxylase enzymes in an uncommon inherited form of hypertension to the much more common essential hypertension?
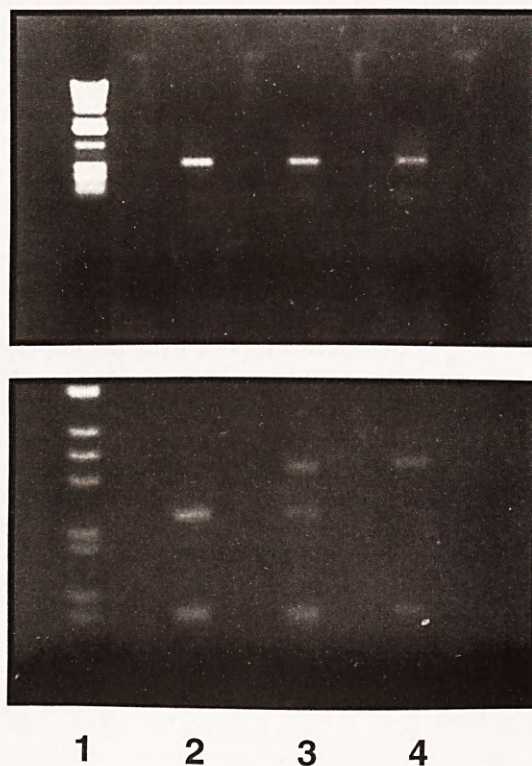
Infusion of ACTH in patients with essential hypertension results in an abnormally high ratio of plasma 11-deoxycortisol to cortisol and of 11-deoxycorticosterone to corticosterone [24]. This suggests that in subjects with essential hypertension the ability of the adrenal cortex to perform the 11β-hydroxylation step is impaired. In addition, the aldosterone response to Ang II infusion is heightened and so is the aldosteronerenin ratio [25].

These biochemical abnormalities could result from one of two mechanisms involving the CYP11B1 and B2 genes. First, abnormal expression of a normal 11β-hydroxylase or aldosterone synthase enzyme could alter their activity in response to endogenous and exogenous stimuli such as ACTH or Ang II, or their level of activity may be set at a different level from that of the normotensive population. Evidence for this is limited but we have detected a polymorphism in the promoter region of CYP11B2 by restriction enzyme digestion of a polymerase chain reaction amplified portion of the promoter region (Fig 5) (A Jamieson, PC White, unpublished data). This polymorphism may affect the regulation of the expression of CYP11B2 in man; studies are underway to determine its effects on the function of CYP11B2 *in vitro*, the frequency of its alleles in normotensive and hypertensive groups, and what effect the alleles have on blood pressure and other variables in adults.

In contrast to aberrant expression of the aldosterone synthase gene, expression of an abnormal aldosterone synthase or 11β-hydroxylase gene may be important in the genesis of essential hypertension. Patients with GSH are hypertensive mainly because they possess the chimaeric CYP11B1/B2 gene. Artificially constructed cDNA plasmids, comprising a 5' portion of CYP11B1 coupled to a 3' portion of CYP11B2, when expressed in COS cells show some aldosterone synthase activity until the plasmid contains exons 1–5 of CYP11B1; that is, the ability to synthesise aldosterone residues in exons 5–9 of CYP11B2 [16] (Fig 6). By altering just a few nucleotides in exon 5 of CYP11B1, the resultant gene product has significant aldosterone synthase activity in addition to its predicted 11β-hydroxylase activity [26].

Fig 5. (a) *PCR product following amplification of the promoter region of CYP11B2.* (b) *Results of digestion of the PCR products with the enzyme HaeIII: 1, size ladder; 2, homozygous for allele 'a'; 3, heterozygote; 4, homozygous for allele 'A'.*
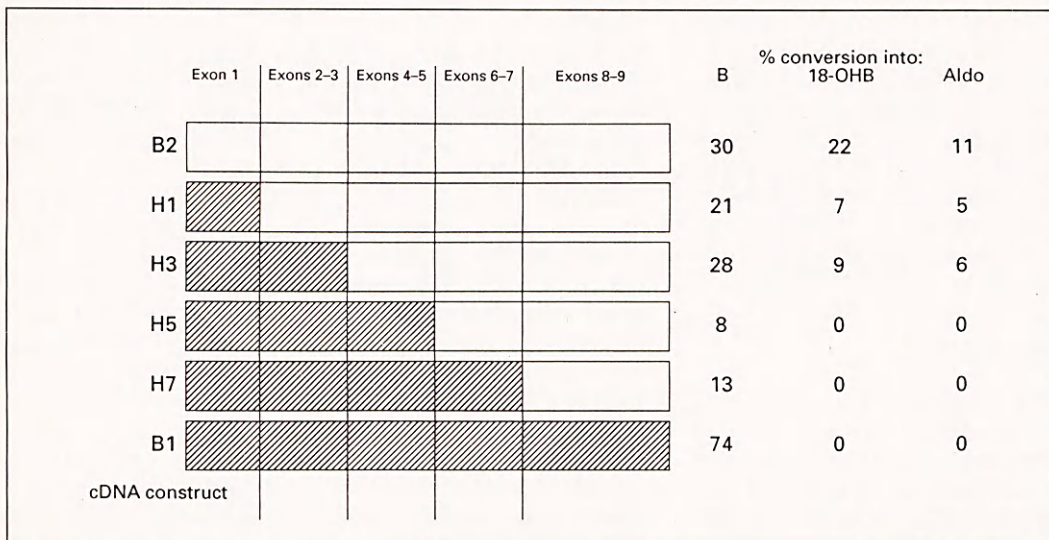

1   2   3   4

	Exon 1	Exons 2–3	Exons 4–5	Exons 6–7	Exons 8–9	B	% conversion into: 18-OHB	Aldo
B2						30	22	11
H1						21	7	5
H3						28	9	6
H5						8	0	0
H7						13	0	0
B1						74	0	0

cDNA construct

Fig 6. *Results of expression of hybrid cDNA plasmids containing CYP11B1 and B2 sequences* in vitro.
Left panel: Plasmids are chimaeric constructs containing variable portions of CYP11B1 and B2: B1, CYP11B1; B2, CYP11B2; H1–7, constructs containing the exons 1–7 of CYP11B1 combined with the remainder from CYP11B2.

Right panel: Percentage conversion of 11-deoxycorticosterone into corticosterone (B), 18-hydroxycorticosterone (18-OHB) and aldosterone (Aldo). Chimaeric plasmids containing the first three exons of CYP11B1 exhibit aldosterone synthase activity which is absent when exons 1–5 are present.
(Adapted from reference 16 with permission).

Conclusion

Hypertension is a complex multifactorial disorder with a clear genetic component. The nature of this genetic influence is not yet fully determined. In GSH, we have the first clear evidence that a single gene disorder inherited in an autosomal dominant manner can cause hypertension. The incidence of GSH is unknown and world-wide only 19 kindreds have been reported [15,16,20,23]. It is likely that its incidence has been underestimated, principally owing to the assumption that hypokalaemia and severe hypertension at a young age are *sine qua non* of the condition; by using genotyping to identify suspected cases a greater number of cases of GSH will be discovered.

Screening populations for GSH by genotyping poses the question 'Who should be screened?' The answer depends to some extent on who is asked. The enthusiast would argue that everyone with hypertension should be screened for GSH and so should all their relatives regardless of their blood pressure because genotyping has identified many normotensive cases of GSH. The sceptic would argue that the condition is a rare cause of secondary hypertension and as such should only be looked for as a 'bottom of the list' cause of refractory hypertension. There is no one correct answer to the question but we recommend a middle-ground approach to screening for the presence of GSH (Fig 7). This approach is likely to detect most people with GSH without wasting valuable time and laboratory resources in screening large populations outwith an epidemiological study.

The study of the molecular biology underlying GSH has stimulated considerable interest in the role of mutations in steroid hydroxylase genes in the development of essential hypertension. Minor changes involving the CYP11B1 and B2 genes may lead to significant

Fig 7. *Guidelines for screening for glucocorticoid-suppressible hyperaldosteronism.*

Patients with hypertension
- Hypertension detected under the age of 25 years
- Hypertension detected in a sib under the age of 25 years
- Family history of stroke under the age of 40 years
- Refractory hypertension in any patient < 60 years old

Special groups
- All patients with evidence of aldosterone excess
- Patients with supressed plasma active renin concentrations (< 3 µg/ml supline; < 5 µg/ml erect) despite normal plasma aldosterone concentrations
- 'Hyperactive families': families where hypertension seems to be prevalent but with no clear mode of inheritance

DNA testing for GSH requires 10 ml DTA preserved blood

changes in the enzymic activity of the resultant gene products. These functional changes could explain the observed physiological abnormalities in some cases of human hypertension—the exaggerated response to Ang II infusion and the apparent impairment of 11β-hydroxylase activity—but further work is required to determine how common this is in essential hypertension.

The task of proving that the CYP11B1 and B2 genes are important in the development of essential hypertension is not a simple one. Three aspects need to be considered when testing whether they play a part in the genetic component of essential hypertension. First, polymorphisms involving the genes must be identified. The chimaeric gene causing GSH is, of course, one such polymorphism with a clear effect on blood pressure. The effect on blood pressure of the polymorphism in the promoter region of CYP11B2 remains to be determined. Other polymorphisms involving these genes have been identified by single-strand conformational polymorphism (SSCP) analysis which allows single base pair differences between different alleles of a gene to be identified according to their mobility on a polyacrylamide gel [27]. This technique is useful for screening the genes of interest, exon by exon, to detect the presence of polymorphisms. In addition to molecular biological know-how, an epidemiological survey of a large population of normotensive subjects is required to determine the incidence of these polymorphisms in the normal population and to allow for the identification of other polymorphisms which occur relatively infrequently in normal populations. Finally, it is necessary to assemble population samples of hypertensive individuals not only to define the incidence of a polymorphism in the hypertensive population but also to provide a population suitable for linkage studies. This latter group would be an affected group of relatives such as sibling pairs with hypertension [28]. Sibling pair group analysis has been particularly useful in showing linkage of polymorphisms of the angiotensinogen gene locus to hypertension and the development of preeclampsia [4–7]. At present we are in the process of performing all three aspects of this study to determine the role of CYP11B1 and B2 in human hypertension.

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NEW TITLE

MY FIRST 75 YEARS OF MEDICINE

by A M Cooke

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