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Genetics of Hypertension
Therapeutic Implications

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Abstract

Essential hypertension affects ≈20% of the adult population, and has a multifactorial origin arising from an interaction between susceptibility genes and environmental factors. The understanding of the molecular basis of essential hypertension may provide us with new and more specific pharmacological agents, and perhaps the ability to individualise treatment and maximise the reduction in risk of morbidity and mortality from cardiovascular disease.

Hypertension due to single gene abnormalities is very rare; however, it follows a Mendelian model of inheritance and therefore can be identified successfully

using family linkage studies. Since clear Mendelian models of inheritance cannot readily be assigned in essential hypertension as there may be variable penetrance of susceptibility genes, other studies with designs based on affected sibling pairs, family-based association studies and case-control studies have been performed.

The renin-angiotensin system (RAS) plays an integral part in the control of blood pressure, and genetic polymorphisms within this system and their effect on the response to antihypertensive therapy are now being studied. Polymorphisms of the angiotensin converting enzyme (ACE) gene, although associated with left ventricular hypertrophy, do not appear to have a clear association with hypertension. Studies on the association of genotype with response to antihypertensive therapy are less consistent for genetic polymorphisms of the RAS. Although some of the results are positive, patient numbers have been small in the studies completed to date.

Genetic polymorphisms of the adrenergic receptors have been associated with blood pressure variation in African-Americans, White Americans and African-Caribbeans. A β_2-adrenoceptor polymorphism exhibits agonist-mediated receptor downregulation which may lead to enhanced peripheral vasoconstriction. Therapeutic studies have not yet been completed on patients with this genotype.

A further polymorphism of the α-adducin gene has been associated with essential hypertension. This may influence blood pressure response to sodium loading/depletion and response to long term treatment with a thiazide diuretic, but further studies are needed to clarify this.

Antisense oligonucleotides targeted against genes of the RAS, e.g. angiotensinogen and the angiotensin type 1 receptor, are being modified to improve targeting and thereby reduce toxicity. However, gene therapy is unlikely to replace pharmacological therapy in the foreseeable future. The immediate goal should be to enhance our understanding of the genetic nature of essential hypertension based on the interaction of genetic makeup with the environment, with a view to individualising antihypertensive therapy.

1. Introduction

Recent advances in molecular genetics such as the polymerase chain reaction which permits amplification or 'photocopying' of tiny amounts of DNA, and the concerted effort of the Human Genome Mapping Project, enable research into the genetic basis of common diseases to become a tangible prospect. In order to realise these goals, a dense map has been developed of genetic markers spanning the 3 billion bases of the human genome. This permits links to be demonstrated between regions of the genome and common human disorders.[1] Such markers exhibit high interindividual variation or polymorphism and may be based on dinucleotide, trinucleotide or tetranucleotide simple sequence repeats (microsatellites), or upon repetitive segments of tens or hundreds of bases known as variable number tandem repeats (minisatellites).[2] In 1997 between 5000 and 7000 such markers were mapped by the Human Genome Mapping Project, thus providing a marker on average every 700 000 base pairs. This equips the molecular geneticist with a suitably dense marker map with which to investigate the genetics of common diseases.

It is now recognised that many common disorders which affect modern society, such as hypertension, arise from complex interactions between genes and environmental factors. The combination of an understanding of the molecular basis of hypertension may provide us with new and more specific pharmacological targets and perhaps, most excitingly, the ability to individualise antihyper-

tensive treatment and maximise reduction in risk of morbidity and mortality from cardiovascular disease. In this article we review progress to date.

2. Essential Hypertension: A Complex Trait

Hypertension is a common condition, affecting approximately 20% of the adult population in Westernised society, and is a significant contributor to morbidity and mortality from cardiovascular disease including stroke, myocardial infarction and end-stage renal disease.[3] Human essential hypertension has a multifactorial origin and is thought to arise from an interaction between susceptibility genes and environmental factors. Twin and family studies which have tested for correlations of blood pressure among relatives suggest that approximately 30% of blood pressure variation arises from genes.[4] With the exception of the rare single gene causes of hypertension where additional biochemical and physiological parameters help to define the link, no clear division is apparent between hypertension and normotension. In fact, blood pressure adopts a normal distribution in the general population and is defined on the basis of thresholds for intervention to reduce blood pressure, and thereby risk of stroke and myocardial infarction.

This pattern of blood pressure distribution in the population suggests that there will be several genes involved in the genetic susceptibility to this multifactorial or 'complex trait'.[1,3] It is possible that genetic variation within an individual gene may have only modest effects on blood pressure unless combined with anomalies within other genes or environmental factors such as sodium in the diet. These inter-relationships may prove difficult to establish for both gene-gene or gene-environment interactions.[1] For example, 2 genetic variations could interact synergistically to elevate blood pressure substantially more than would be expected from the individual effect of the variants; alternatively, the interaction could be additive, placing an additional increment on blood pressure.

Normal blood pressure is maintained by the physiological interaction of cardiac output and peripheral resistance, and by the control of salt and water balance by the kidney. Despite identification and understanding of the physiological systems involved in the regulation of blood pressure, it remains unclear which systems are causative in essential hypertension.

3. Genetic Strategies for Hypertension

Traditional linkage studies are based upon entire pedigrees but they require specification of a Mendelian model of inheritance, and the demonstration of tracking of genetic variation through the family with the trait of interest.[1] The analysis is based upon a logarithmic odds ratio (LOD score) which expresses the likelihood of linkage divided by the likelihood of nonlinkage.[1] A LOD score of 3.0 is conventionally accepted as evidence in favour of linkage and means that the odds are 1000 to 1 in favour of linkage rather than nonlinkage of a genetic marker.[1] Such studies have been successful in rarer hypertensive syndromes where a clear Mendelian model of inheritance can be specified, and hypertensive traits may be identified by a specific response to drugs, e.g. corticosteroids in glucocorticoid-remediable aldosteronism, or an associated biochemical phenotype, e.g. hypokalaemia in Conn's syndrome.

4. Hypertension Due to Single Gene Abnormalities

4.1 Glucocorticoid-Remediable Aldosteronism

Glucocorticoid-remediable aldosteronism (GRA) is an autosomal dominant form of moderate-to-severe hypertension associated with both an excess of cerebral haemorrhage and Celtic ancestry.[5] The hypertension is caused by excessive secretion of aldosterone, and possibly additional adrenal mineralocorticoid hormones, where aldosterone secretion is regulated by corticotrophin [adrenocorticotrophic hormone (ACTH)] rather than by angiotensin II.[6] Kindreds with GRA

demonstrate a novel gene on chromosome 8 that represents duplication arising from unequal crossover between the aldosterone synthase and 11 β-hydroxylase genes, such that the regulatory sequences of 11 β-hydroxylase are fused with coding sequences of aldosterone synthase.[6] Aldosterone synthase gene expression and enzymatic activity are therefore brought under the control of ACTH. The chimeric gene results in ectopic production of aldosterone with hypertension due to increased salt and water retention.[6] Plasma renin activity and angiotensin II production are decreased; aldosterone remains under ACTH control and is therefore not suppressed.

Identification of the mechanism for this rare Mendelian hypertension explains the reduction of blood pressure in response to administration of physiological doses of glucocorticoids which suppress ACTH secretion and thereby suppress expression of the mutant gene.[6] It is now possible to define these individuals using a simple genetic test rather than extensive biochemical phenotyping. This is the first example of such an approach in clinical practice to identify hypertensive individuals who should receive glucocorticoids as antihypertensive therapy.

4.2 Syndrome of Apparent Mineralocorticoid Excess

The syndrome of apparent mineralocorticoid excess is an autosomal recessive disorder that produces moderate-to-severe hypertension of early onset with very low levels of aldosterone.[7] The hypertension is caused by stimulation of the mineralocorticoid receptor by cortisol, which circulates at levels that are orders of magnitude higher than those of aldosterone. Since cortisol has equal affinity for the mineralocorticoid receptor there is, in normal circumstances, a protective mechanism which operates in the distal convoluted tubule whereby the enzyme 11 β-hydroxysteroid dehydrogenase type 2 metabolises cortisol to cortisone. Cortisone is incapable of stimulating the mineralocorticoid receptor and this prevents cortisol from acting at the mineralocorticoid receptor, resulting in selective activation by aldosterone.[7] Mutations in the gene cloning 11 β-hydroxysteroid dehydrogenase type 2 leading to loss of enzyme activity have been confirmed in patients with apparent mineralocorticoid excess.[8,9] Insufficient 11 β-hydroxysteroid dehydrogenase to metabolise cortisol has also been proposed to explain the hypertension seen in Cushing's syndrome and in glucocorticoid resistance caused by mutations in the glucocorticoid receptor.[3]

4.3 Liddle's Syndrome

Liddle's syndrome is characterised by early presentation of moderate-to-severe hypertension with hypokalaemia, suppression of the renin-angiotensin system (RAS) and low aldosterone levels.[10] Hypertension is caused by enhanced renal reabsorption of salt and water. Aldosterone antagonists are ineffective but reduction in blood pressure and correction of hypokalaemia by triamterene or amiloride suggested an abnormality of the distal epithelial sodium channel.[10] This amiloride-sensitive epithelial sodium channel comprises 3 subunits: an α subunit which supports sodium conductance, and β and γ subunits which govern the activity of the channel. Patients with Liddle's syndrome have mutations in the genes encoding either the β or γ subunits which produce mutant subunits leading to a marked increase in sodium reabsorption in the distal convoluted tubule.[11]

4.4 Pseudohypoaldosteronism Type II (Gordon's Syndrome)

This autosomal dominant form of hypertension and hyperkalaemia, which is very responsive to thiazide diuretics, has recently been mapped to 2 distinct sites on chromosomes 1 and 17.[12,13] This suggests there may be more than one cause for this disorder, which may include a renal ion channel abnormality.[13] In this context it is interesting that rat and human comparative mapping studies have identified a locus on the long arm of chromosome 17 which maps to the same region as one of the loci for Gordon's syndrome.[14] As yet the precise cause of Gordon's syndrome has not been defined; how-

ever, identifying the abnormality would be beneficial as a marker of thiazide drug responsiveness.

5. Human Essential Hypertension

The single gene disorders confirm the importance of genetic influence on blood pressure. However, the approaches outlined above have been successful because a clearly defined hypertensive phenotype could be identified and a clear pattern of inheritance could be assigned. This type of model-specific pedigree analysis is less likely to be successful in the general trait of essential hypertension where clear Mendelian modes of inheritance cannot readily be assigned because there may be variable penetrance of susceptibility genes.[1] In addition, age of onset of the trait may be variable, or there may be an absence of environmental stimuli or other gene interactions necessary to disclose the phenotype.[1]

The heterogeneity of potential genetic mechanisms and environmental influences for hypertension, coupled with interindividual variability in antihypertensive efficacy, particularly among ethnic groups,[15] has prompted researchers to ensure homogeneity in the study populations of individuals with essential hypertension. In some of the larger programmes currently under way this has led to identification of individuals who, despite having a lean body build with low levels of alcohol (ethanol) consumption, are severely hypertensive, and are representative of those with maximal genetic influence upon blood pressure.

6. Study Design for Genetic Studies in Essential Hypertension

There are several strategies for study design which could prove effective to determine the genetic basis of hypertension, given the potential problems with traditional pedigree-based analysis.

6.1 Model-Free (Nonparametric) Linkage

Model-free (nonparametric) linkage relies only on allele sharing by first-degree relatives, as opposed to parametric linkage which requires precise specification of the mode of trait inheritance. In this approach, affected sibling pairs are usually studied.[1] If genetic variation at a locus plays a role in determination of a phenotype, then the affected siblings will share alleles or genetic variation at that locus more often than is predicted by chance.[16] These methods can cope with variable penetrance and do not require parental genotype, which is an advantage in disorders of late onset like hypertension.[1] However, this approach may raise statistical power issues as many areas of the genome may be searched for cosegregation of alleles; therefore, a correction must be made for multiple comparisons.[1,17] Accordingly, it has been argued that a stringent significance level for a definite nonparametric linkage should be set at $p < 0.00001 \times 2.2$, to reduce the risk of false-positives.[16] Large collections of affected sibling pairs would therefore be needed to offer adequate power to fulfil these criteria.[17]

6.2 Discordant Sibling Pair Analysis

Discordant sibling pair analysis has been advanced as a powerful approach for quantitative traits.[18] The power of such studies is critically dependent upon the definition of families with extremely discordant sibling pairs and the demonstration of tracking of 'hypertensive genetic variation' with the trait.[18] This study design, however, is laborious as families with extreme discordance for blood pressure within them need to be identified.

6.3 Association Studies

Association studies are used to define risk in unrelated individuals. Genetic markers are tested in unrelated cases of hypertension rather than family members. The frequency of a mutation or polymorphism is compared between hypertensive individuals and matched controls. Approximately 100 to 1000 cases and controls are required, depending on factors such as the frequency of the polymorphism, and the difference it is desirable to detect between groups.[17] The case-control study is a necessary step in determining the usefulness of

markers detected in linkage studies. To be relevant, a genetic marker must demonstrate linkage disequilibrium, i.e. it must be so close to a disease mutation that both remain virtually inseparable through the generations from the common ancestor.[17] There are risks in the case-control study design in that selection bias or unanticipated ethnic heterogeneity may give spurious results, and results should therefore be treated cautiously until replicated.[1]

6.4 Family-Based Association Studies

Family based association studies may be very useful in minimising the risks of selection bias and will become widely applied in the future.[1] This approach is essentially used once the genetic locus of interest has been defined and involves tracking distortion in transmission of the parental genotype to affected offspring, using unaffected family members as controls.[1] The transmission distortion test requires knowledge of the parental genotype and for the parents to be heterozygous for the markers of interest, i.e. to possess both versions of a genetic variant which enables testing for the distortion of allelic transmission to the affected offspring.[1] In the case where parents are not available, multiple siblings must be used to work out the parental genotype. This novel use of within-family controls has been applied to assist in resolving the role of the insulin gene locus in type 1 (insulin-dependent) diabetes.[19]

7. The Candidate Gene Approach, Genome Screens and Genetic Polymorphisms of the Renin-Angiotensin System

Candidate genes are selected from systems physiologically implicated in blood pressure regulation, e.g. the RAS. Ideally, it may be possible to demonstrate biochemical or functional differences in the gene product which track with blood pressure through families, and also to identify genetic variation that associates with the trait.

Genome screening utilises a dense map of highly polymorphic markers which are spread evenly throughout the human genome to test for linkage of chromosomal regions to, in this case, blood pressure.[20] The potential advantage of this approach is that no prior hypothesis is formulated regarding any specific candidate system.[21] This provides the opportunity to identify chromosomal regions which harbour susceptibility genes that are not suspected to contribute to the hypertensive phenotype.[21] There is a possibility that such an approach may yield new systems for pharmacological manipulation and further enhance the repertoire of treatments for hypertension. There are several programmes attempting genomic screening in cardiovascular diseases at this time.

The RAS controls systemic and intrarenal blood pressures and sodium balance, and is an important target for antihypertensive therapy. Several polymorphisms have been identified within genes encoding the RAS which may contribute to the development of elevated blood pressure, and which may influence therapeutic response to blood pressure–lowering therapy.

8. The Angiotensinogen Genotype, Hypertension and Response to Therapy

The rate limiting step in the formation of the vasoactive hormone angiotensin II is the cleavage of angiotensinogen by renin. A variant of the gene encoding angiotensinogen results from a substitution of threonine for methionine (Met→Thr) at position 235 (*M235T*), and this polymorphism may be a marker for blood pressure variation. Linkage of the angiotensinogen gene locus to hypertension has been reported in sibling pairs of White European origin and African-Caribbeans.[22-24] In addition, an association has been demonstrated between the *T235* allele and both plasma angiotensinogen concentration and elevated blood pressure variation.[22,25] However, other studies have not replicated this finding. This is probably due to heterogeneity of hypertension, which may mean different variants of the same gene locus are important in different hypertensive individuals.[23,24,26]

Recently, in a further study of 10 polymorphisms within the angiotensinogen gene, it was

found that one particular combination of the *T235* allele and a polymorphism, located just next to the initial transcription site, which changes a guanine to adenine at position -6, was most strongly related to hypertension.[27] Subsequently, this variant has been shown in Japanese hypertensive patients to alter levels of angiotensinogen by altering the transcription rate within the regulatory region of this gene.[28,29] This emphasises the need to consider all variations within a gene to define combinations or haplotypes which may alter blood pressure.

In the context of the conflicting results which have been reported on association between angiotensinogen genotype and blood pressure variation, it is perhaps hardly surprising that analyses of the angiotensinogen genotype and blood pressure response to antihypertensive therapy are not consistent. There was no association between angiotensinogen genotype and blood pressure reduction by a calcium antagonist (n = 63), β-blocker (n = 79) and angiotensin converting enzyme (ACE) inhibitor (n = 91) in unrelated West European Caucasians treated for 4 weeks in a placebo-controlled, crossover design.[30] Plasma angiotensinogen, angiotensin II and renin activity were not measured.

In another study, angiotensinogen genotype was strongly associated with blood pressure reduction after 5 weeks' ACE inhibition (n = 125). Although plasma angiotensinogen was not measured in this study, the best blood pressure response was associated with the *T235* allele.[31] Furthermore, in a population-based group of Caucasians, the *T235* allele was associated with the use of antihypertensive treatment – in particular, the risk of developing hypertension at a younger age and requiring combination antihypertensive therapy.[25] Emerging evidence suggests that a variation in the regulatory sequence of angiotensinogen may be more important in evaluating the response to blood pressure–lowering treatment.[28,29]

9. The ACE Genotype, Hypertension and Response to Therapy

The ACE gene contains an insertion/deletion polymorphism (I/D) which depends on the presence or absence of a 250 base pair DNA fragment. This polymorphism correlates with plasma ACE activity which is higher in those with the deletion (DD) allele.[30,32,33] Studies of the association between ACE genotype and variation in blood pressure are conflicting. A significant association between hypertension and the ACE insertion (II) allele has been reported.[31,34] However, no association has been reported in several other study groups.[33,35-37]

In spite of the absence of mortality studies using ACE inhibitors in hypertension, the efficacy of these agents in reducing mortality from left ventricular dysfunction has generated enthusiasm for investigating whether antihypertensive response can be predicted.

In one parallel study in patients with essential hypertension the ACE polymorphism was determined before 15 days' treatment with either an ACE inhibitor (enalapril), a calcium antagonist (verapamil) or a β-blocker (bisoprolol). The ACE polymorphism was associated with response to antihypertensive therapy, where enalapril (n = 17) produced a greater reduction in blood pressure in the DD variant and verapamil (n = 17) produced a more consistent decrease in blood pressure in the II variant. In addition, there was a significant reduction in plasma angiotensin II levels and a significant increase in plasma renin activity with ACE inhibition in the DD variant alone without an effect on aldosterone levels. Treatment with verapamil produced a significant increase in plasma renin activity in all 3 genotypes without an effect on angiotensin II or aldosterone levels.[32] While there was no comment on blood pressure control in relation to genotype after β-blocker therapy (n = 16), there was also no effect of β-blockade on plasma renin activity, angiotensin II or aldosterone levels. However, patient numbers were very small (n = 5 for genotype) in this study and the method of blood pressure measurement was not described; therefore, these result must be treated with caution.

In contrast, 2 larger studies found no association between ACE genotype and response to antihypertensive therapy. The first study compared 4

weeks' treatment with nifedipine (n = 63), atenolol (n = 79) and lisinopril (n = 91), using ambulatory blood pressure monitoring (ABPM).[30] The second study examined ACE inhibition alone for 5 weeks (lisinopril, captopril, enalapril, perindopril, n = 125) where blood pressure was recorded using a semi-automated device.[31] Similarly, in a Japanese study there was no difference between ACE genotype in blood pressure response after treatment with enalapril for 1 year (n = 60). However, there was significantly greater improvement in left ventricular hypertrophy and impaired diastolic function in hypertensive patients with the DD genotype (n = 10), although once again numbers were small.[33]

10. The Angiotensin Type 1 (AT_1) Receptor Genotype, Hypertension and Response to Therapy

The angiotensin type 1 (AT_1) receptor mediates the haemodynamic effects of angiotensin II. A silent polymorphism where cytosine is substituted for adenine ($A^{1166} \rightarrow C$) at position 1166 of the AT_1 receptor gene has been associated with severe essential hypertension.[31,38] Furthermore, a significant interaction between the ACE and AT_1 receptor gene loci in terms of influence on blood pressure variation was reported, the mechanism of which is unclear.[31]

A significantly higher expression of the AT_1 receptor has been reported in normotensive (n = 30) and hypertensive (n = 50) individuals in association with the ACE DD genotype.[32] In addition, AT_1 receptor expression was higher in hypertensive patients than in normotensive volunteers, and a positive correlation with plasma renin activity was observed for both groups ($r = 0.72$, $p < 0.01$; $r = 0.66$, $p < 0.05$, respectively).

The AT_1 receptor genotype was not associated with blood pressure response to ACE inhibition (n = 125).[31] Although numbers were small, there was significant reduction in AT_1 receptor expression in patients with the DD allele (n = 5) when treated with an ACE inhibitor, and in patients with the II allele (n = 5) when treated with a calcium antagonist.[32]

10.1 The AT_1 Receptor Genotype and Aortic Stiffness

The same silent polymorphism *A1166C* which has been associated with raised blood pressure may be an independent influence on aortic stiffness. Carotid femoral pulse wave velocity as a measure of arterial compliance may be a surrogate for hypertensive arterial damage.[39] Since ACE inhibitors are thought to improve arterial compliance, the influence of perindopril on aortic stiffness was compared with nitrendipine in patients according to AT_1 receptor genotype.[40] Patients in the perindopril group who were homozygous for *C1166* exhibited a 3-fold greater reduction in pulse wave velocity than those in the other treatment groups.[40] This represents an interesting but early attempt to evaluate the influence of antihypertensive therapy on a surrogate end-point by genotype.

11. The Sympathetic Nervous System, Genetic Variation and Hypertension

The sympathetic nervous system influences cardiac output, vascular tone, renal sodium reabsorption and renin release, and could be implicated in enhanced vascular responsiveness observed in some hypertensives. Such an effect could arise from genetic variants which may alter the agonist response of α-adrenoceptors, leading to enhanced vasoconstriction, attenuation of β_2-adrenoceptor–mediated vasodilation or, alternatively, altered central sympathetic drive or signal transduction pathways.

Genetic variation in either the α-adrenoceptor leading to enhanced vasoconstriction, or the β_2-adrenoceptor leading to attenuated vasodilation, may be an important mechanism for enhanced peripheral resistance. In a recent study, polymorphisms of the β_2- and α_2-adrenergic receptors were significantly associated with blood pressure variation. The β_2-adrenergic receptor was associated with African-Americans and White Americans and the α_2-adrenergic receptor locus was associated

with White Americans only.[41] Since no functional effect of these polymorphisms has been defined, we can only speculate as to the mechanism.

An amino terminal variant which encodes glycine (Gly) instead of arginine (Arg) at position 16 (Arg 16→Gly 16) within the β_2-adrenoceptor exhibits exaggerated agonist mediated receptor downregulation, and could therefore lead to enhanced vascular reactivity. An association between the *Gly16* pro-downregulatory variant, using an allele-specific polymerase chain reaction, and blood pressure variation has been observed in African-Caribbeans.[42] It is possible that the restriction fragment length polymorphism (RFLP) alleles reported by Sevetkey et al.[41] are in linkage disequilibrium, or close physical proximity, to the *Gly16* variant. These observations may account for the increased total peripheral resistance observed in African-American hypertensive patients and might result from enhanced downregulation of the β_2-adrenoceptor due to the β_2-adrenoceptor genotype.

Further support for impaired vasodilation in people of African ancestry emerges from observations of blunted forearm vasodilation, which has been reported in African-Americans in response to infusion of the β_2-adrenoceptor agonist isoprenaline (isoproterenol).[43] This blunting of response was not due to presynaptic release of noradrenaline (norepinephrine) leading to vasoconstriction, as there was no evidence of a significant increase in noradrenaline spillover. It is entirely possible that the genotype at the β_2-adrenoceptor may explain this phenomenon but this remains to be proven in studies where individuals identified for genotype have forearm blood flow measured in response to β_2-adrenoceptor stimulation.

12. α-Adducin Genotype and Hypertension

Studies in Milan hypertensive and normotensive rats suggest mutations within a gene encoding a part of the cytoskeleton, α-adducin, which may alter renal tubular sodium reabsorption.[44,45] In the rat model, mutations within this gene account for 50% of the observed difference in blood pressure between hypertensive and normotensive strains.[44] Since there are strong similarities between humans and rodents for the α-adducin genotype, this represents an important candidate for study in humans.

Studies in 86 human hypertensive sibling pairs revealed support for linkage of the chromosomal region containing the α-adducin gene to high blood pressure.[46] A polymorphism which exchanges tryptophan for glycine at position 460 (*G460T*) in the gene product associates with hypertension and evidence suggests that this polymorphism may influence the response of blood pressure to sodium loading or depletion. In addition, *G460T* polymorphism may influence response to long term therapy with a thiazide diuretic.[46] Patients who were heterozygous for the polymorphism exhibited a greater blood pressure response to sodium depletion and long term therapy with a thiazide than those who were homozygous for the glycine variant.[46] If these results are reproducible in further studies, this may represent a way of predicting a diuretic-responsive form of hypertension.

13. G Protein Variant Alters Sodium Exchange

Recent evidence suggests that sodium hydrogen exchange is enhanced in hypertensive individuals.[47] The observation that this is preserved in cell lines with increased intracellular calcium mobilisation and DNA synthesis points to an anomaly in signal transduction as the cause.[48] Subsequently, it has been reported that the guanine nucleotide binding regulatory proteins linked to the sodium hydrogen exchanger may be altered. The gene encoding a specific β subunit known as GNB3 harbours a mutation where cytosine is swapped to thymidine at position 825. This truncates the final GNB3 protein which would normally be expected to reduce activity of the channel.[48] In contrast, those homozygous for the thymidine variant with the deletion exhibit substantially enhanced sodium hydrogen exchange.[48] This variant of GNB3 has recently been associated with hypertension in a large case-control study.

Such abnormalities within the signal transduction pathway could present new targets for pharmacological action which makes this particularly exciting.

14. Gene Therapy for Hypertension?

Gene therapy is under investigation for a number of single gene disorders, malignancies and in AIDS. In order to justify development of gene therapy, researchers have focused upon disorders where there is a severe disease phenotype and the gene defect has been established.[2] Although in hypertension there are conventional drug therapies available which are effective in reducing the risk of stroke and heart attack, some researchers are exploring gene therapy, which they argue may be more attractive if the antihypertensive action remains effective for weeks or months.[49] To date, gene therapy has focused on antisense technology, where short lengths of targeted nucleic acid (oligonucleotides) are used to switch off or reduce levels of gene product by preventing mRNA transcription or translation.[49] Such experimental work suggests that after delivery of naked antisense oligonucleotides to blood or brain this approach may effectively reduce plasma levels of angiotensinogen or AT_1 receptor, and hence blood pressure, over 3 to 7 days in animal models.[49] The disadvantages of such technology include problems with specificity of action, efficacy of cellular uptake, stability and longevity of activity and low toxicity.

In order to improve upon direct injection of nucleic acid, delivery using cationic liposomes encapsulating antisense oligonucleotide against angiotensinogen has been tested. Although this was efficient there was no direct comparison with administration of naked antisense oligonucleotide.[50] In addition, limited experiments have been conducted on viral vector delivery of antisense oligonucleotide. In this context, a viral vector must be genetically modified to prevent the vector causing disease and should not cause mutation of host cells or an inflammatory response. Adenovirus tends to cause inflammation and retroviral vectors may cause mutagenesis; they are, therefore, only useful in experimental hypertensive models. However, the adeno-associated virus is not associated with an inflammatory response, and has been used to transfer antisense oligonucleotide to the AT_1 receptor in the spontaneously hypertensive rat with promising results over a 9-week period.[51]

Currently, it is difficult to predict whether gene therapy for human hypertension will become viable; were it to become so, cost might be prohibitive. It seems more plausible that genetic understanding of hypertension will be employed to develop predictions of pharmacological response and perhaps newer antihypertensive therapies.

15. The Future for Hypertension Genetics and the Prediction of Treatment Response

It is likely that significant progress toward the genetic basis of complex diseases such as hypertension will be made in the next few years. It would seem that at such a critical phase in our understanding of this disorder, we should be considering how we will design suitable studies to test whether new diagnostics predict treatment response. Anyone conducting a mortality or morbidity study should consider storage of DNA to evaluate relationships between genotype and end-points or genotype and therapeutic response. Recent technological advances such as high throughput genotyping provide the capability to generate rapid results. However, as can be seen from some of the confusion surrounding treatment response and genotype within the RAS, we will need to carefully design studies of adequate size, which minimise selection bias, and ensure that genotypes are identified according to treatment response.[17]

A group of researchers is currently using pharmacological response to therapy to define homogeneous groups of hypertensive patients for study of genotype/phenotype relations. Over several years patients have been randomised to the main classes of antihypertensive agents in order to test the relationship between drug response and genotype.[17] This might provide an opportunity to focus on specific hypertensive subtypes, and in the future may

represent a useful way of defining which antihypertensive agents will be most effective in which hypertensive patients.

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