MOLECULAR GENETICS OF SALT-SENSIVITY AND HYPERTENSION

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ABSTRACT:

For the past decade, hypertension research has shifted strongly in the direction of molecular genetics. The success stories are the monogenic hypertensive syndromes. Classic linkage analyses have located the responsible genes for glucocorticoid-remediable aldosteronism, Liddle syndrome, and apparent mineralocorticoid excess. Furthermore, a recent gain-of-function mutation has recently been described in the gene for the mineralocorticoid receptor. These genes have been cloned and their functions elucidated. Other monogenic syndromes are currently being intensively studied. However, in the area of primary hypertension, the successes have relied on the candidate gene approach. Allelic variants in the genes for angiotensinogen, α-adducin, the β2-adrenergic receptor, the G-protein β3-subunit, and the T594M mutation in the β-subunit of the epithelial sodium channel have been identified; however, the importance of these allelic variants to primary hypertension as a whole is not yet clear. Recently, an association approach was employed to implicate the mineralocorticoid receptor gene in salt-sensitivity. Linkage approaches have been attempted and the β-subunit of the epithelial sodium channel has been linked to hypertension and to blood pressure as a quantitative trait locus. New approaches are necessary to elucidate salt-sensitive hypertension. The analysis of multiple genes simultaneously in terms of a metabolic control analysis may provide a more promising approach.

Guyton and colleagues (1990) developed a cybernetic framework to define the phenomenon of pressure-natriuresis in terms of a series of differential equations. Their model had multiple interconnecting junctions showing the relationship between short-term, long-term, and infinite gain systems controlling blood pressure. Their model was extremely useful to explain the fact that all organisms relying on sodium and water metabolism to regulate their internal environment exhibit a relationship between salt and water intake (as well as excretion) and blood pressure. In normal individuals, this relationship is extremely steep. We showed, 25 years ago, that normotensive persons must ingest up to 800 mmol/day of sodium and chloride to exhibit an increase in blood pressure (Luft et al., 1979). We observed that African Americans had a greater increase in blood pressure at high levels of sodium intake, compared with whites. This observation suggested that blacks might be more salt-sensitive (in terms of blood pressure responses) than whites. This observation, coupled with the finding that first-degree relatives of patients with hypertension excreted salt in an attenuated fashion compared with persons who had no hypertensive relatives, suggested to us that salt-sensitivity might have a genetic basis (Luft et al., 1987). We performed studies in monozygotic and dizygotic twin subjects and found that not only was blood pressure influenced by genetic variance, but also that intermediary phenotypes, such as glomerular filtration rate, renal blood flow, excretion of sodium and potassium after acute volume expansion, renin, aldosterone, and plasma catecholamine values were also influenced by genetic variance. Today, we are in a position to identify genes responsible for regulating salt and water balance as well as blood pressure. A considerable amount of progress has already been made, particularly in terms of monogenic disorders.

Monogenic Hypertension

Monogenic hypertension is the bright spot in the area of molecular genetics of human hypertension. The attitude here is that by elucidating rare monogenic diseases, we shall come to understand mechanisms of disease applicable to primary hypertension (Luft et al., 1995). This promise has been kept largely through the efforts and successes of Lifton and colleagues. Glucocorticoid-remediable aldosteronism is a good example (Lifton et al., 1992).

Glucocorticoid-Remediable Aldosteronism

Patients with glucocorticoid-remediable aldosteronism have an autosomal-dominant monogenic hypertension and are usually suspected of having primary aldosteronism. They have a volume expansion, salt-sensitive, form of hypertension, tend to metabolic alkalosis with hypokalemia (not invariably), and respond to both thiazide diuretics and spironolactone. The latter fact is a clinical clue that mineralocorticoid products may be involved. Their renin values are low whereas the aldosterone values are both elevated. The patients also have 18-hydroxy and 18-oxocortisol in their urine, steroids not normally found in appreciable amounts. Recognizing these abnormal products (an intermediate phenotype) led to solving the mystery. Replacement amounts of prednisone ameliorate the hypertension, cause the abnormal steroids to disappear, and give the syndrome its name. The abnormal cortisol derivatives and the favorable effects of glucocorticoid treatment suggested that inner cortical zones, which express the gene for 17α-hydroxylase (CYP17) and are adrenocorticotropin-responsive, were the source of the excess mineralocorticoids. Two distinct gene products (11β-hydroxylase and aldosterone synthase) perform the terminal steps in glucocorticoid and mineralocorticoid
biosynthesis, respectively. A linkage analysis in a large pedigree localized the responsible gene to chromosome 8, exactly at the site where the genes for 11β-hydroxylase and aldosterone synthase also reside (Lifton et al., 1992). This fact suggested that a chimeric gene might be responsible, which indeed proved to be the case. Aldosterone synthase (CYP11B2) and 11β-hydroxylase (CYP11B1) reside on chromosome 8. In affected individuals, a chimeric gene consisting of the promoter- regulatory region of CYP11B1 and the structural portion of CYP11B2 is located between CYP11B2 and CYP11B1. The protein product resulting from this gene performs all reactions required for aldosterone production, thus causing adrenocorticotropin-dependent hyperaldosteronism. Ectopic expression of the chimeric protein in the inner cortical zones, which also express CYP17, permits the formation of 18-hydroxy and 18-oxocortisol, the biochemical hallmarks of glucocorticoid-remediable aldosteronism. Finally, suppressing steroidogenesis in the zona fasciculata and reticularis with exogenous glucocorticoids alleviates the hypertension. The chimeric gene results from a mitotic mismatch and unequal crossing over. In all instances, the crossover is located 5’ to intron 4 of the CYP11B genes.

**Apparent Mineralocorticoid Excess**

Genetic apparent mineralocorticoid excess (AME1) resembles the syndrome observed in persons ingesting large amounts of licorice. Licorice gluttony and treatment with carbenoxolone both cause a volume expansion, low renin, low aldosterone, and salt-sensitive form of hypertension, which may also feature metabolic alkalosis and hypokalemia. Interestingly, the hypertension responds to both thiazide and spironolactone, but no abnormal steroid products are present in the urine. Both licorice and carbenoxolone contain glycyrrhetinic acid, which was found to inhibit the enzyme 11β-hydroxysteroid dehydrogenase. 11β-Hydroxysteroid dehydrogenase is responsible for converting cortisol to cortisone. In the distal renal tubule, this step is crucial for protecting the mineralocorticoid receptor, which has the same affinity for cortisol as it does for aldosterone. This step protects us all from developing AME. Inhibition of 11β-hydroxysteroid dehydrogenase results in AME. Interestingly, AME may also occur as a rare, autosomal-recessive form of monogenic hypertension. The 11β-hydroxysteroid dehydrogenase gene, which has a renal-specific renal isoform, was a hot candidate gene for this condition. The clinical clues helpful in resolving this condition were: volume-dependent salt-sensitive hypertension, tendency to hypokalemia and metabolic alkalosis, low renin and low aldosterone values, responsiveness to both thiazides and spironolactone, despite absence of aldosterone or any abnormal mineralocorticoid products, and resemblance to licorice gluttony. Mune et al. (1995) solved the mystery. In eight of nine families, mutations in the renal-specific isoform gene for 11β-hydroxysteroid dehydrogenase were found, which indeed rendered the product incapable of converting cortisol to cortisone. Thus, the mineralocorticoid receptor is unprotected from cortisol in these patients, and cortisol functions to occupy the mineralocorticoid receptor. The fascinating possibility that AME might be relevant in the heterozygous state has been raised by Li et al. (1997), who observed a patient with apparent mineralocorticoid hypertension at age 38 years, who had a daughter with homozygous AME. The patient had low renin and aldosterone concentrations and was found to have a mutation in the gene for 11β-hydroxysteroid dehydrogenase.

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1 Abbreviations used are: AME, apparent mineralocorticoid excess; ENaC, epithelial sodium channel; ACE, angiotensin-converting enzyme; AGT, angiotensinogen.

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**Liddle Syndrome**

Liddle described patients with autosomal-dominant monogenic hypertension who also tended to metabolic alkalosis with hypokalemia. His patients had low renin and low aldosterone values; however, they did not respond to spironolactone, although thiazides and triamterene reduced the blood pressure. This observation convinced Liddle that they probably did not have a form of mineralocorticoid excess. Liddle speculated that they would show a distal tubular defect of enhanced sodium and chloride reabsorption. A renal transplant performed on a patient with Liddle syndrome who developed renal failure cured the disease, providing strong evidence that the problem resided within the kidneys rather than in a regulatory system (Botero-Velez et al., 1994). Shimkets et al. (1994) subsequently localized the responsible gene of a family with Liddle syndrome to chromosome 16 and were able to show that the gene encodes for the β-subunit of the epithelial sodium channel (ENaC). The channel is amiloride- and triamterene-sensitive, explaining the efficacy of these drugs in the syndrome. The channel remains inappropriately permeable even in the face of high salt intake (Fig. 4), thereby explaining the salt-sensitive hypertension. Subsequently, a mutation in the γ-subunit of ENaC has been found, which can also result in Liddle syndrome (Hansson et al., 1995). The molecular mechanisms of Liddle syndrome involves alteration or deletion of a PY motif in the cytoplasmic tails of the β- or γ-subunits. Consequently, Nedd4 binding fails to occur, the channels are not internalized and instead remain activated on the cell surface (Palmer and Alpern, 1998).

**Activating Mutation in the Mineralocorticoid Receptor**

Geller and colleagues (1999) recently described a new form of human hypertension caused by an activating mutation in the mineralocorticoid receptor. They observed heterozygous persons with a mutation substituting leucine for serine at position 810. The mutation is close to the hormone-binding domain and proved to be of great functional importance. The receptor appears to function in an aldosterone-independent fashion. During pregnancy, female patients develop an even greater increase in blood pressure, suggesting that occupancy of the receptor with progesterone increases its activity further, rather than attenuating activity. The same holds true for spironolactone treatment, which aggravates hypertension in this condition.

**Antimatter**

Analogous to the presumed existence of “black holes” and “anti-matter”, one might speculate that the reverse of such a syndrome could also exist. Indeed, mutations in the subunits of ENaC were found to cause relative hypotension and salt wasting with hyperkalemic acidosis (pseudohypoaldosteronism type I). Mutations in either the α- or the β-subunit, inherited in an autosomal-recessive manner, result in loss of channel activity, thereby explaining the pathophysiology of the disease (Chang et al., 1996). Recently, Geller et al. (1998) have described autosomal-dominant pseudohypoaldosteronism type I. The phenotype of autosomal-dominant pseudohypoaldosteronism type I, renal salt wasting with dehydration, hypotension, hyperkalemia, and metabolic acidosis, is similar to that of the ENaC mutations, with the exception that it remits with age. The condition involves mutations in the mineralocorticoid receptor gene. Other channel gene mutations can also result in blood pressure, salt, and water regulatory diseases. For instance, Gitelman syndrome is a variant of Bartter syndrome and features inherited hypokalemic alkalosis, hypomagnesemia, and hypocalciuria. The syndrome is caused by mutations in the thiazide-sensitive Na-Cl cotransporter (Simon et al., 1996c). Clin-
ically, the patients look like individuals who surreptitiously are ingesting thiazide diuretics. Bartter syndrome can be caused by several different mutations, including genes for the Na-K-2Cl cotransporter (Simon et al., 1996a), the outwardly directed potassium channel ROMK (Simon et al., 1996b), and the chloride channel gene CLCNKB, on the basolateral cell surface (Simon et al., 1997). The above syndromes are examples of monogenic “hypotension”; however, they are relevant to hypertension nonetheless.

The gene(s) responsible for pseudohypoaldosteronism type II have recently been mapped, albeit not yet cloned. Pseudohypoaldosteronism type II features familial hyperkalemia and was first described by Gordon et al. (1970). Thiazide diuretics are highly effective in this syndrome, commensurate with salt-sensitivity. A multilocus linkage analysis yielded a lod score of 8.1 for linkage to chromosomes 1q31-q42 and 17p11-q21 (Mansfield et al., 1997). Interestingly, the chromosome-17 locus overlaps a syntenic interval in the rat that contains a blood pressure quantitative trait locus. Pseudohypoaldosteronism type II provides promise in leading to cloning of two additional as yet not appreciated genes leading to hypertension.

**Autosomal-Dominant Hypertension with Brachydactyly**

An additional promising monogenic syndrome is autosomal-dominant hypertension with brachydactyly. This condition features salt-resistant hypertension. We mapped a gene for hypertension to the short arm of chromosome 12 (12p) in a large Turkish kindred with hypertension and type E brachydactyly. In this family, the phenotypes hypertension and brachydactyly always are inherited together; they cosegregate 100% (Schuster et al., 1996a). Affected persons are shorter than nonaffected individuals and do not have volume expansion-induced hypertension as determined by a volume expansion and contraction protocol, but instead resemble patients with essential hypertension (Schuster et al., 1996b). The mechanism of the hypertension is unknown. Thus far, this syndrome has only been described in this Turkish kindred and a similar family in Canada. Recently, we encountered another such family in the United States. In these three families, the hypertension also follows an autosomal-dominant mode of inheritance and cosegregate 100% with short stature and type E brachydactyly (Toka et al., 1998). A deletion syndrome in a Japanese child with type E brachydactyly, as well as the additional families, has enabled us to sharply decrease the area on 12p containing the gene (Bähring et al., 1997); however, a four million-base pair segment remains, and we have not yet cloned the gene.

**Primary Hypertension**

Literally, a thousand papers have been published on this topic and only the highlights will be mentioned here. A brief overview of papers in 1997 and 1999 on patients with primary hypertension revealed research on the following genes: angiotensin-converting enzyme (ACE), angiotensinogen, β2-adrenergic receptor, α-adducin, angiotensinase C, renin-binding protein, G-protein β3-subunit, atrial natriuretic peptide, insulin receptor, and endothelial nitric-oxide synthase in hypertension of pregnancy. The ACE gene has generally not been associated with hypertension despite the obvious effectiveness of ACE inhibitors; however, there are exceptions. Even telomere length has been raised as being important to primary hypertension. Despite their interest, discussion of all these genes is beyond the scope of this commentary; however, in my view six genes, ACE, angiotensinogen, α-adducin, the β2-adrenergic receptor, G-protein β3-subunit, and the T594M mutation in the β-subunit of the epithelial sodium channel are of particular relevance.

### Angiotensin-Converting Enzyme

The ACE gene locus was linked to blood pressure in spontaneously hypertensive rats in 1991, and although the ACE gene insertion/deletion allelic variant has been implicated in arteriosclerotic cardiovascular disease, cardiac hypertrophy, restenosis, progression of diabetic renal disease, and progression of IgA nephropathy, hanging a guilty verdict in terms of hypertension onto the ACE gene has been difficult (Soubrier, 1998). O’Donnell et al. (1998) found evidence for association and genetic linkage of the ACE gene with hypertension and blood pressure in men, but not in women, when they analyzed over 3000 participants from the Framingham Heart Study. The data were significant, but not robust. Fornage et al. (1998) studied 583 three-generation pedigrees from Rochester, MN and were able to show that variations in a microsatellite marker within the growth hormone gene, which is close to the ACE gene locus, influenced interindividual blood pressure differences in young white men, but not in women.

### Angiotensinogen

Jeunemaitre et al. (1992) first reported linkage of the angiotensinogen (AGT) gene locus to hypertension in hypertensive siblings from France and Utah. Subsequent screening identified the so-called AGT 235T variant in hypertensive cases as being more frequent in hypertensive cases than in controls. The variant is associated with higher AGT levels and appears to be in tight linkage disequilibrium with a promoter mutation –6 base pairs (G-6A) upstream of the initiation site of transcription (Inoue et al., 1997). This mutation may result in a higher basal transcription rate. The haplotype combining the AGT 235T and G-6A polymorphisms appears as the ancestral allele of the human AGT gene and as the one associated with hypertension (Jeunemaitre et al., 1997).

Caulfield et al. (1996) have investigated AGT extensively and reported linkage of the AGT locus to blood pressure in 77 European families ($p < 0.003$). Their studies in African Caribbeans supported the notion that the AGT locus is linked to hypertension. Since the initial reports, many studies have been published on the association between allelic variants in AGT and hypertension. Kunz et al. (1997) have recently reviewed the evidence on AGT 235T from 11 studies of 14 populations. Data on 5493 patients showed that the AGT 235T allele was significantly associated with hypertension (OR 1.2, CI 1.11–1.29). These data were significant statistically; however, their clinical significance is another matter. The authors concluded that much more than AGT 235T was responsible for primary hypertension. The AGT gene has been the most scrutinized and the most promising finding of the primary hypertension genes thus far; however, the AGT 235T variant explains only a relatively small part of blood pressure variance.

### α-Adducin

To my knowledge, α-adducin is the only example of rat molecular genetic research contributing pertinent information to the molecular genetics of human hypertension. A mutation in rat α-adducin was found responsible for 50% of the hypertension in the Milan hypertensive rat. The mutation was shown to be responsible for an increase in Na-K pump activity in renal cell transfection experiments. Linkage and association studies were subsequently performed in hypertensive patients and controls and a point mutation (G460W) was found in the human α-adducin gene. The 460W variant was shown to be more frequent in hypertensive patients than in controls. The pressure-natriuresis relationship was subsequently studied in 108 hypertensive patients. The relationships suggested a shifted, reduced-slope, salt-
sensitive pressure-natriuresis curve in persons bearing the W variant. The α-adducin studies combine molecular genetics and physiology in rats and patients and present a truly remarkable story of careful observations, patience, and scholarship, which has been summarized elsewhere (Manunta et al. 1998). The importance of α-adducin to other hypertensive populations and to salt-sensitive hypertension must await additional studies. Recently, a Japanese group (Kato et al., 1998) was unable to find an association between α-adducin allelic variants and essential hypertension.

**β2-Adrenergic Receptor**

A restriction fragment length polymorphism in the β2-adrenergic receptor gene was associated with and linked to salt-sensitive hypertensive persons of African origin in earlier studies (Svetkey et al., 1997). An amino terminal variant in the β2-adrenoceptor, which encodes glycine instead of arginine at base pair position 46 (Arg-16 → Gly), has been described and appears to have functional significance (Yang-Feng et al., 1990). The variants showed equal affinity for epinephrine or isoproterenol; however, the Gly-16 variant exhibited increased down-regulation in response to isoproterenol, compared with the Arg-16 variant (Green et al., 1994). Such a down-regulation pattern could lead to impaired vasodilatory responses to circulating β2-adrenergic agonists. This hypothesis is supported by in vivo studies showing that pulmonary β2-adrenoceptors with the Gly-16 variant also exhibit increased down-regulation in response to salbutamol, compared with the Arg-16 variant. Furthermore, a recent report indicating that the Gly-16 variant in the β2-adrenoceptor is associated with nocturnal asthma renders further support to the notion that this polymorphism may have major functional importance (Turki et al., 1998). The importance of α-adducin to other hypertensive populations and to salt-sensitive hypertension must await additional studies. Recently, a Japanese group (Kato et al., 1998) was unable to find an association between α-adducin allelic variants and essential hypertension.

**T594M Mutation in the β-Subunit of the Epithelial Sodium Channel**

A variant of the β-subunit of the amiloride-sensitive sodium channel was described by Su et al. (1996), who also observed increased channel activity in lymphocytes in African Americans. Baker et al. (1998) recently studied 206 hypertensive black patients and 142 normotensive black control subjects in London, UK. Seventeen (8%) of the hypertensive blacks had the T594M mutation, compared with 2% of normotensive blacks. Persons with the mutation had lower plasma renin activity, supporting the notion of increased sodium reabsorption. Thus, the T594M mutation may serve to explain some degree of salt-sensitivity and hypertension in blacks. The elucidation of Liddle syndrome led to the discovery of this allelic variant. The finding underscores the potential relevance of rare monogenic diseases to complex genetic disease.

**Apparent Mineralocorticoid Excess as Primary Hypertension**

Mutations in 11-bHSD2 have already been discussed in terms of monogenic hypertension. Recently, additional mutations were described by Morineau et al. (1999), namely the A328V and the R213C mutations. An intermediate phenotype of this condition is the urinary excretion of 5α- and 5β-tetrahydrometabolites. The relationship between cortisol and cortisone metabolites can be compared. An increased excretion of cortisol metabolites and a decreased excretion of cortisone metabolites would reflect diminished activity of 11-bHSD2. Lovati et al. (1999) have tested this hypothesis. They found that normotensive salt-sensitive men phenotyped with a dietary protocol excreted more 5α- and 5β-cortisol metabolites, whereas salt-resistant men excreted more 5α- and 5β-cortisone metabolites. Their findings suggest that variants in 11-bHSD2 might contribute to primary salt-sensitivity of blood pressure. They tested this hypothesis by means of a biallelic marker in the gene. Indeed, they were able to associate variants of this marker with salt-sensitivity and salt-resistance. This finding may be an important clue to salt-sensitivity.

**Challenge for the Future**

Although major efforts have been expended, excellent experiments have been performed, and exciting stories have been told, the results in the area of human molecular genetics of hypertension are modest. In terms of genetically explaining blood pressure variance for specific genes, we have a long way to go. The above seven genes and their allelic variants are worthy of special discussion, in my view, because of the thought processes involved in their evaluation. Linkage analysis was employed in the case of three of the gene variants, namely for the AGT, α-adducin, and β2-adrenergic receptor gene. However, we cannot conclude that these genetic variants were found by linkage analysis. These genes were candidate genes that were selected by investigators and then subjected to a linkage analysis. That the genes of the renin-angiotensin system and the genes for catecholamine receptors, and genes for the enzymes involved in their production and degradation might be involved in hypertension, would have occurred to students of hypertension 50 years ago. α-Adducin and the G-protein β3-subunit were identified as candidates by whole animal and cell physiology approaches, which resulted in their being selected as candidate genes. A linkage analysis was subsequently performed in hypertensive sibling pairs and the α-adducin gene locus was indeed linked to hypertension. Two linkage studies have shown that the β-subunit of the epithelial sodium channel is linked to blood pressure as a quantitative trait locus, respectively (Nagy et al., 1999; Wong et al., 1999).

The use of biallelic single nucleotide repeat markers and the inten-
tion to create a map of 300,000 single nucleotide repeats across the genome will surely radically affect genotyping plans in elucidating the genetics of hypertension. It will become increasingly important to put genetic information in the context of what has long been known about how enzymes, channels, transporters, etc. behave and how they are regulated. Without such a framework, there is little chance of predicting the effects of mutations, deletions, or insertions of genes. Such an approach has been termed a metabolic control analysis (Bowden, 1999). Guyton’s framework from the 1970s, expanded to include more information on regulatory systems, could be well adapted to such a strategy. In terms of salt-sensitive hypertension, it will become more important to examine variability in groups of genes operating in a given network, rather than analyzing single polymorphisms separately. After all, to understand a whole, we must examine the whole.

References


