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MINIREVIEW

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Development of genetic hypotheses in essential hypertension

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Abstract Essential hypertension illustrates the formidable task presented by the identification of genetic determinants of common disease. Making an initial genetic inference may prove difficult enough; the subsequent demonstration of functional significance at various levels of biological integration may be even more challenging. We review three instances in which an initial genetic inference has led to the development of testable hypotheses pursued at increasingly higher levels of biological organization. These include the adducin, the G protein β3 subunit, and the angiotensinogen hypotheses.

Key words Essential hypertension · Genetics · Mechanism of disease · Adducin · G protein β3 subunit · Angiotensinogen

Introduction

The regulation of arterial pressure involves the integration of a large number of biological systems that control vascular structure and tone, body fluid volume and composition, and their adaptation to constantly changing physiological needs. The work of leading physiologists has established that sustained elevation in arterial pressure could be brought about by either one of two primary mechanisms, namely, general vasoconstriction, including the renal arteries, or enhanced plasma volume due to increased sodium retention (Guyton 1980). While the concept points to a major source of etiological heterogeneity in essential hypertension, the compensatory adaptation of various regulatory systems masks the primary alteration initiating the pathological process. Effective therapy rests on targeting this initial determinant of disease. If genetic differences underlie individual responses, then genetics offers an opportunity to identify these primary targets.

Four major genetic paradigms are being applied that involve either animal models or human subjects. The animal models of hypertension include the development and analysis of hypertensive rat strains and the manipulation of the expression of specific genes by transgenic methods. Genetic approaches in humans encompass molecular investigations of rare inherited syndromes of human hypertension, and direct analysis of essential hypertension in humans. Each has recognized advantages and limitations that have been reviewed (Corvol et al. 1989; Lifton 1996; Rapp 1991; Smithies et al. 2000). Various leads in the genetics of essential hypertension have appeared in the literature, as reviewed by Luft (1998). We will provide an overview of three instances in which genetic hypotheses have been subjected to direct tests among patients with essential hypertension and have opened investigative avenues on putative underlying mechanisms at the molecular, cellular, organ, and organismal levels. These include the α-adducin, the G-protein β3 subunit, and the angiotensinogen hypotheses. Genetic inference and the refinement of the initial hypothesis will be reviewed here, with primary reference to the more significant advances in each line of investigation. While we will review the natural progression of the work, we will also provide in each instance a diagrammatic recapitulation of the findings or hypotheses developed at the various levels of biological integration defined in Fig. 1.

Adducin

In work led by Bianchi and colleagues over three decades, the progressive refinement of the understanding of a rat model of hypertension, the Milan hypertensive rat (Barber et al. 1994), was recently extended to human essential hypertension. On the basis of a considerable amount of experimental data generated almost exclusively by this group,

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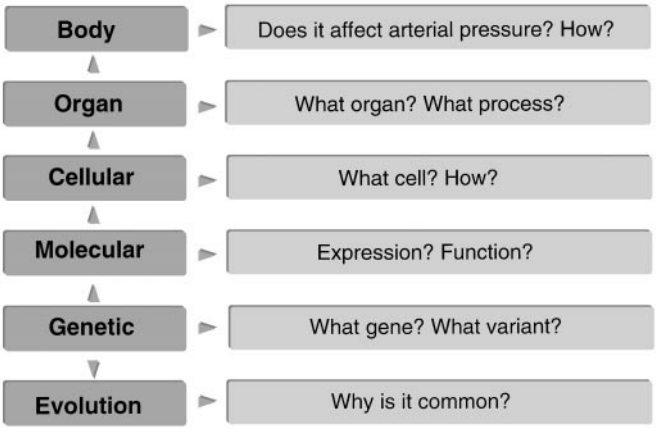

Fig. 1. Genetic determination at various levels of biological integration

a very specific hypothesis has developed which proposes a molecular and physiological mechanism for essential hypertension, with attendant diagnostic and therapeutic implications. Simply stated, the hypothesis is that individual differences in the gene encoding α-adducin account for sodium sensitivity and predisposition to essential hypertension through enhanced sodium reabsorption in proximal tubule. The development of the hypothesis follows a methodical journey that began with the generation of the Milan hypertensive (MHS) and normotensive (MNS) strains, investigation of their renal physiology, focus on the Na^+-K^+ ATPase activity, genetic cosegregation, indictment of α-adducin, and identification of mutations differentiating MHS from MNS, and, ultimately, forays into human hypertension through linkage, association, physiological, and cellular investigations.

This line of research began with the establishment of hypertensive and normotensive strains (MHS and MNS) from two pairs of Wistar-derived founders in 1965. The animals were bred through, primarily, brother-sister mating, and selected for divergent systolic arterial pressure. By 1994, the strains had been maintained through 70 generations of inbreeding and had a coefficient of co-ancestry of 0.23. The hypertension in this model is mild, with mean systolic pressures of 169 ± 4 and 131 ± 3 mmHg in MHS and MNS, respectively (Barber et al. 1994).

The primary role of the kidney in this model was established through classical cross-transplantation experiments involving normotensive MNS and pre-hypertensive or hypertensive MHS animals as either donors or recipients (Bianchi et al. 1973, 1974). Subsequent physiological and hormonal studies provided a refined characterization of functional differences between MHS and MNS animals, as recapitulated by Barber et al. (1994). The most significant observations were higher glomerular filtration rates for both whole kidney and single nephron (Boberg and Persson 1986), blunted tubuloglomerular feedback response to volume expansion, higher water and urine output, lower urine osmolality, lower plasma vasopressin, and lower plasma renin concentration in pre-hypertensive MHS animals compared with findings in MNS. Ancillary experiments and the changes observed as hypertension developed led Bianchi and colleagues to conclude that, in the pre-hypertensive stage, MHS animals exhibited a transient state of volume expansion due to enhanced reabsorption in proximal tubule. As hypertension and increased peripheral resistances came into play, the differences in renal function disappeared.

These conclusions set the search for a molecular mechanism that could account for a primary defect in sodium handling. Through a variety of subsequent experiments in vivo and in vitro (Ferrandi and Bianchi 2000), suspicion centered on increased activity of the Na^+-K^+ ATPase (Ferrandi et al. 1996) and endogenous ouabain-like factors that may interact with the pump (Ferrandi et al. 1992). This work, in turn, led to the hypothesis that enhanced basolateral activity of this transporter in proximal tubule could account for sodium-dependent hypertension. On the assumption that the underlying molecular determinant was ubiquitous, the investigation was extended to other cell types. In erythrocytes, significant differences in Na^+-K^+ co-transport between the two strains were reported that cosegregated with arterial pressure in appropriate genetic crosses (Bianchi et al. 1985).

Additional experiments, and the emerging significance of the cytoskeleton in the regulation of cell volume and membrane transport activities, led the authors to test whether a genetic difference between MHS and MNS animals was associated with membrane or cytoskeleton. This set the stage for cross-immunization experiments between the two strains, using either erythrocyte membrane or cytoskeleton preparations. The treatment of MHS animals with cytoskeleton extracts from MNS animals led to the production of antibodies against a 105-kDa protein (Salardi et al. 1989) that proved to be the adducin protein (Gardner and Bennett 1986). Mutations were thereafter identified in both the α- and β-subunits of this heterodimeric protein (Tripodi et al. 1991), which accounted for 50% of the difference in systolic blood pressure between MHS and MNS (Bianchi et al. 1994). The functional relationship between adducin, its polymorphisms, and Na^+-K^+ ATPase activity was demonstrated through subsequent in-vitro experiments (Tripodi et al. 1996), with evidence of direct interaction between the two proteins (Ferrandi et al. 1999).

The hypothesis that a similar mechanism could be operative in human hypertension was tested through association and linkage. The initial finding consisted of an association between multiallelic markers around the α-adducin locus and essential hypertension in a case-control study (Casari et al. 1995). Of several multiallelic markers tested, those closest to the α-adducin locus exhibited the most significant association. In a subsequent report, significant linkage in 137 pairs of hypertensive siblings (Cusi et al. 1997) provided additional support for the genetic hypothesis. A common molecular variant was identified that encoded the substitution of tryptophan for glycine at residue 460 of the protein (G460W), with an allelic frequency of 0.13–0.16 in control subjects. This variant was significantly associated with hypertension in two case-control comparisons, involving

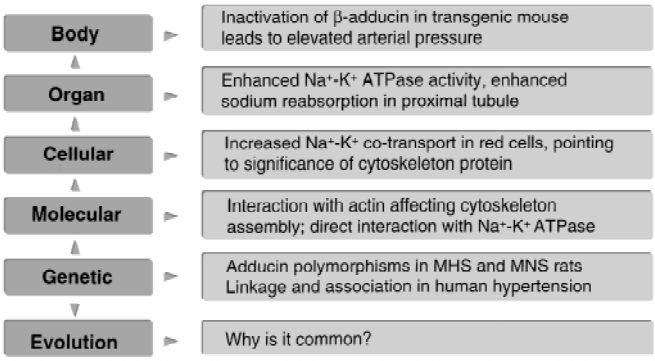

Fig. 2. The adducin hypothesis. *MHS*, Milan hypertensive; *MNS* Milan normotensive (rat strains)

Italian and French subjects, respectively. The acute response of arterial pressure to sodium depletion induced by diuretic administration was examined as a function of genotype in 86 hypertensive patients. Carriers of the G460W variant exhibited a significantly greater drop in arterial pressure than noncarriers. Chronic response to sodium depletion was investigated in a similar manner in 58 patients. In addition, carriers of the G460W variant had significantly lower plasma renin activity (Cusi et al. 1997). The hypothesis that enhanced sodium reabsorption in proximal tubule could account for human hypertension was supported by evidence of increased fractional excretion of both endogenous lithium and uric acid by patients with the G460W variant (Manunta et al. 1999).

Overall, the work constitutes a unique example of the progression of a pathogenic and genetic hypothesis developed in animal models toward specific inferences on the genetic and physiological basis of essential hypertension in humans. For both the Milan hypertensive rat and essential hypertension in humans, the thesis is that variation in the α-adducin molecule induces significant differences in activity of the Na^+-K^+ ATPase, which, in proximal tubule, affects sodium reabsorption. Enhanced sodium reabsorption in G460W carriers promotes a low renin, volume-dependent form of essential hypertension. Control of this enhanced activity by a digitoxigenin-like derivative, PST 2238, has been proposed as an effective antihypertensive treatment on the basis of in-vitro and in-vivo experiments in the Milan hypertensive strains (Ferrari et al. 1998). The observation of hypertension in mice in which the β-adducin gene has been inactivated by gene targeting (Marro et al. 2000) provides additional support for the hypothesis that mutations affecting the adducin protein can affect baseline arterial pressure. A summary of the progress achieved at various levels of observation is provided in Fig. 2.

G protein β3 subunit

The incrimination of the gene encoding the G protein β3 subunit in essential hypertension and obesity has developed along a classical "forward" pathway of inference, proceeding from the biochemical delineation of a phenotype at the cellular level toward the definition of candidate genes and, ultimately, the identification of a molecular variant associated with both the cellular phenotype in cultured cells and essential hypertension in case-control comparisons.

Following various leads that suggested increased activity of the Na^+/H^+ exchanger in various cells or tissues of hypertensive patients compared with findings in controls, Siffert and colleagues concentrated their effort on cultured lymphoblastoid cell lines obtained from normotensive controls and hypertensive patients (Rosskopf et al. 1993). Nine cell lines, referred to as NT lines, were generated from normotensive males with a negative family history of hypertension and in whom low Na^+/H^+ exchanger activity had been consistently recorded in both platelets and lymphocytes. Ten cell lines, denoted HT, were derived from hypertensive males with at least one hypertensive parent and elevated Na^+/H^+ exchanger activity in similar experiments. Although persistent differences in the activity of this exchanger were observed when six cell lines of each type were contrasted, no mutation could be detected in the gene encoding the ubiquitous exchanger, NHE-1, that is responsible for this transport (Rosskopf et al. 1993). As a result, the focus shifted to signal transduction upstream from NHE-1. Subsequent work with these cells (Siffert et al. 1995) documented significant differences in platelet-activating factor (PAF)-mediated proliferation, and rises in cytosolic free calcium and in inositol phosphate formation between NT and HT cells. Significant differences were also observed in guanosine triphosphate (GTP)γS formation — a quantitative assay of G protein activation — after stimulation by either PAF or MAS-7, a peptide that mimics an activated G-protein-coupled receptor. The inhibition of these enhanced signals by pertussis toxin (PTX) (Siffert et al. 1995) and the stimulation from agonists that activate PTX-sensitive G proteins (Pietruck et al. 1996) suggested the involvement of a subset of G proteins.

G proteins are heterotrimeric molecules that mediate stimuli from a large variety of membrane receptors, and their subunits are encoded by a large number of genes. After an initial search through several of these genes proved negative (Pietruck et al. 1996), screening of the gene encoding the β3 subunit (*GNB3*) in two NT and two HT cell lines led to the identification of a genetic polymorphism at position 825 of the cDNA sequence (Siffert et al. 1998). Five NT cell lines tested were all homozygous for the C allele, whereas, of six HT cell lines tested, one was homozygous CC, one heterozygous TC, and the remaining four were homozygous TT.

The mechanism relating the C825T substitution to functional alteration of *GNB3* was not immediately evident. The substitution, occurring in exon 10 of the gene, does not change the serine encoded at this site. A shorter mRNA species, denoted *Gβ3-s*, was identified in cell lines carrying the 825T alleles, however. This shorter form, not detected in any clones derived from three cell lines homozygous for the CC genotype, was observed in 139 of 498 clones derived from five cell lines with either the CT or the TT genotypes. Subsequent analyses of a large number of clones showed

that, while full-length mRNA could be generated from either C or T alleles, *Gβ3-s*, with few exceptions, originated from the T allele. Sequence analysis of *Gβ3-s* indicated that this species resulted from activation of a cryptic acceptor splice site in exon 9 of the gene, leading to the formation of a truncated protein lacking 41 amino acids.

How the 825T allele could affect splicing at such a distant site remains unknown. Evidently, 825T may be a marker in strong linkage disequilibrium with an unidentified variant located in the intervening intronic sequence. The shorter introns 7 and 8, and splice donor and acceptor of intron 9, showed no additional variants. Both the full-length and the *Gβ3-s* variants appeared fully functional when expressed in Sf9 insect cells, and the authors offered a model affording maintenance of folding and function (Siffert et al. 1998).

An association between 825T and hypertension was tested in a case-control study including 427 hypertensive and 426 normotensive German subjects ascertained in the three cities of Berlin, Essen, and Heidelberg. The difference in frequency was significant at the 0.008 level (Siffert et al. 1998). As expected of case-control comparisons, the significance of this association was variable in subsequent replication attempts, as discussed by Siffert (2000).

Further studies have revealed a strong association between 825T and obesity (Siffert et al. 1999b), as well as the nephropathy of type II diabetes (Bluthner et al. 1999). Although the mechanism relating 825T to enhanced G protein and NHE-1 activity in cultured cells appears well understood, the physiological mechanism by which this cellular phenotype relates to hypertension, obesity, or diabetic nephropathy remains a matter of active investigation. It is remarkable that 825T exhibits marked worldwide variation in frequency (Siffert et al. 1999a) in a manner consistent with a thrifty genotype hypothesis. In developing this hypothesis almost four decades earlier, James Neel (Neel 1962) provided a model that could account for the high prevalence of genetic determinants of common disease in modern-day populations by emphasizing the lag of genetic evolution in the face of rapid cultural change; genetic variants once of adaptive value had become deleterious with environmental change. The allelic frequency of 825T was around 0.30 in Caucasians, 0.40–0.50 among Chinese and Japanese, and reached 0.80–0.90 in African populations.

Refined analysis of the extent of polymorphism in the gene, and either functional tests or tests of association involving other variants may provide a clearer picture regarding both molecular mechanism and associations with clinical phenotypes in various populations. In a recent report (Rosskopf et al. 2000), the authors described several additional variants, including the substitution of thymine for cytosine in the 3′ untranslated region of the gene. Remarkably, the 825T allele almost always occurred with the 1429T variant in German subjects, but the relationship between the two variants did not exhibit parallel variation among major ethnic groups. The findings and hypotheses that have arisen from this research are summarized in Fig. 3.

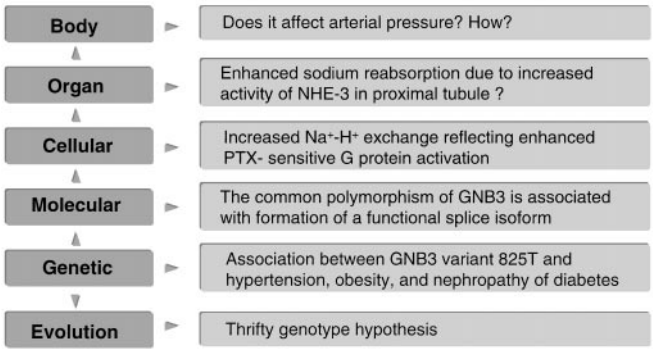

Fig. 3. The G protein β3 subunit hypothesis. *PTX*, Pertussis toxin; *NHE*, Na^+/H^+ exchanger

Angiotensinogen

The work on the angiotensinogen (*AGT*) gene has developed along a typical candidate gene, reverse genetic approach, in the sense that the inference proceeds from gene to function, by contrast with the classical forward approach illustrated by the GNB3 investigations. The *AGT* work stemmed from the well established evidence for a the central role of the renin-angiotensin system (RAS) in arterial pressure regulation.

Genes encoding components of the system were tested by linkage analysis in pairs of hypertensive siblings. Through collaborative efforts involving Utah and French scientists, linkage tests were applied in two large series of Caucasian hypertensive siblings ascertained in Salt Lake City and Paris. No linkage was indicated for either renin (Jeunemaitre et al. 1992b, and unpublished data) or angiotensin-converting enzyme (ACE) (Jeunemaitre et al. 1992a). For angiotensinogen, by contrast, significant linkage was observed in both series, with greater significance achieved when analysis was restricted to more severe hypertensive pairs (Jeunemaitre et al. 1992c).

Linkage to angiotensinogen suggested the hypothesis that individual variants could be found that would be more common in hypertensive probands than in normotensive controls. Coding regions, splice junctions, and proximal promoter were screened for the presence of polymorphism. A common variant, encoding the presence of either threonine or methionine at residue 235 of the mature protein, exhibited significant association with essential hypertension in case-control comparisons involving both the Utah and the French samples (Jeunemaitre et al. 1992c). The association achieved greater significance when only more severe hypertensive probands were considered. The allele occurring at higher frequency among hypertensive subjects, T235, was also significantly associated with elevated plasma angiotensinogen concentrations in both samples.

Subsequently, a significant association between T235 and hypertension was also observed in a case-control study involving Japanese subjects (Hata et al. 1994). Because estrogenic states, including pregnancy, lead to marked elevation of plasma angiotensinogen and increased inci-

dence of hypertension, the M/T(235) polymorphism was examined in preeclampsia, a form of pregnancy-induced hypertension in which a genetic component has long been suspected. In both Caucasians and Japanese, T235 occurred at significantly higher frequency in women with preeclampsia than in women who experienced normal pregnancy outcomes (Ward et al. 1993). As for the previous two hypotheses presented, replication attempts achieved or failed to reach significance, but two subsequent meta-analyses (Kunz et al. 1997; Staessen et al. 1999) support significance in the aggregate. The reason for lack of replication and the inherent limitations of the case-control design have been often discussed elsewhere (Corvol et al. 1999).

The inference derived from these studies, statistical as it was, provided no functional clue. In particular, whether T235 is causally involved or is only a marker for one or more functional variants in the gene remains unsettled. No differences were observed in either the rate of renin cleavage, the stability, or the rate of secretion of the protein when both forms were transiently expressed in cultured mammalian cells (Inoue et al. 1997), but other experiments suggested that the variant, occurring in the vicinity of a cysteine, may affect angiotensinogen complex formation and reactivity with renin (Gimenez-Roqueplo et al. 1998). An unexpected finding was that a common variant in the proximal promoter of the gene, the presence of an adenine instead of a guanine six residues upstream from the initiation site of transcription, was in quasi-complete linkage disequilibrium with the (M/T)235 polymorphism. With few exceptions, all T235 alleles carried the −6(A) variant, while all M235 alleles exhibited −6(G) at this position (Inoue et al. 1997). DNA binding experiments with crude nuclear protein extracts and transactivation assays involving segments of the *AGT* promoter linked to a reporter consistently indicated that nucleotide variation at −6 could affect *AGT* transcription. This in-vitro inference could not be directly extended to the in-vivo function of the gene. Two lines of argument support the hypothesis that individual differences in gene expression may exist in vivo, however. Smithies and colleagues introduced a novel gene titration transgenic approach in the mouse that allowed them to demonstrate that arterial pressure and plasma angiotensinogen increased as the number of *AGT* genes was varied from one to four (Kim et al. 1995; Smithies and Kim 1994). When the expression of *AGT* T235 and M235 alleles was quantified in placental tissues of 39 heterozygous women, a significantly higher expression of the T235 allele over its M235 counterpart was observed (Morgan et al. 1997).

The common occurrence of other variants of *AGT* adds another degree of complexity to attempts to establish causal relationships between genetic variation and predisposition to hypertension. Indeed, new variants have been identified in the *AGT* proximal promoter that also appear to be of functional significance, at least as can be tested in vitro. The substitution of C for T 67 nucleotides downstream from transcription initiation (Ishigami et al. 1999; and our unpublished observations) also appears to affect specific interaction with nuclear proteins and transcriptional activation, as tested in cultured cells (manuscript in preparation). Allele C67 is also in complete linkage disequilibrium with alleles −6(A) and T235. Another variant occurring at nucleotide −20 affects interaction with nuclear proteins and in-vitro expression in a manner that could be gender-specific (Zhao et al. 1999). An ongoing investigation of the entire *AGT* genomic sequence in an extended series of subjects has unraveled a large number of other variants, several of which appear in strong disequilibrium with those previously identified (Nakajima and colleagues, manuscript in preparation). If common variants exist, it is conceivable that more than one of these may modulate angiotensinogen expression or function, thereby generating a complex relationship between genotype and phenotypic expression.

The identification of molecular variants accounting for moderate differences in gene expression or in protein function is proving, not unexpectedly, rather challenging. The difficulty is further compounded by the fact that *AGT* is expressed in multiple tissues. Liver expression contributes the substrate for a systemic, endocrine RAS, while expression in other tissues is an element of various local RAS. The functional overlap and likely elements of coordinated regulation of these systems present additional challenges in attempts to elucidate the physiological mechanism by which individual variation in *AGT* may impact on propensity to develop hypertension. An oversimplified perspective can be developed describing the effects of angiotensin II (A-II) in terms of two contrasts, namely, systemic versus renal and acute versus chronic. Systemic A-II affects vascular tone acutely and vascular structure chronically. Renal A-II, by contrast, may affect renal hemodynamics acutely and sodium reabsorption chronically.

The evidence that intrarenal A-II is a an important regulator of arterial pressure in response to dietary sodium (Hall and Brands 1993) and the expression of angiotensinogen in proximal tubule (Ingelfinger et al. 1986) led us to examine expression of RAS components in the kidney and to propose that a tubular RAS may function in a paracrine fashion along the entire nephron (Rohrwasser et al. 1999). Angiotensinogen, although not filtered through the glomerular membrane, is synthesized and secreted into luminal fluid of the proximal tubule where, by interacting with filtered renin of systemic origin and ACE at the luminal surface of the tubule, it may lead to A-II formation that regulates sodium reabsorption in this proximal nephron segment. While systemic renin is released by the afferent artery of the glomerulus, we found that renin was also synthesized and secreted in the connecting tubule (CNT). Synthesis at this strategic site of convergence of multiple nephron segments forces the consideration that CNT renin may act on luminal angiotensinogen originating in proximal tubule to contribute to the regulation of sodium transactions in terminal segments of the nephron (Rohrwasser et al. 1999). The significance of this paracrine system in plasma volume regulation is under investigation.

Independent evidence has been obtained suggesting that angiotensinogen expression in proximal tubule and its interaction with filtered renin at this site may affect arterial

pressure regulation. This was achieved by the generation and analysis of mice expressing two transgenes (Davisson et al. 1999). The first transgene results in testosterone-inducible overexpression of human angiotensinogen restricted to proximal tubule by the use of a tissue-specific promoter. The second transgene leads to general overexpression of human renin. Animals carrying either of the transgenes singly exhibited normal arterial pressure, whereas doubly-transgenic animals responded with marked pressure elevation upon induction of human angiotensinogen in proximal tubule.

The significance of the kidney in mediating the effects of individual differences in angiotensinogen on arterial pressure is supported by direct observations in humans. The blunted response to infused A-II under conditions of high dietary sodium that appear to distinguish a subset of hypertensive patients (Williams et al. 1996), was significantly associated with homozygosity to T235 (Hopkins et al. 1996).

Other clinical correlations have been reported in population-based epidemiological studies. In the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) Augsburg cohort study, the T235 allele was associated with a substantial proportion of subjects with antihypertensive drug use (Schunkert et al. 1997). In a study of 1509 Caucasians participating in phase II of the Trials of Hypertension Prevention (TOHP), angiotensinogen genotype was associated with significantly greater arterial pressure reduction in response to sodium restriction in a 3-year prospective follow-up (Hunt et al. 1998). A randomized trial of low-sodium mineral salt revealed significant differences in arterial pressure reduction as a function of *AGT* genotype (Hunt et al. 1999). In the Dietary Approaches to Stop Hypertension (DASH) trials, homozygous carriers of the T235 alleles exhibited significantly greater arterial pressure reduction than other genotypes in study subjects consuming either a "fruit and vegetable diet" or this diet combined with reduced fat intake (Svetkey et al. 2000). The progressive refinement of the angiotensinogen hypothesis is described in Fig. 4.

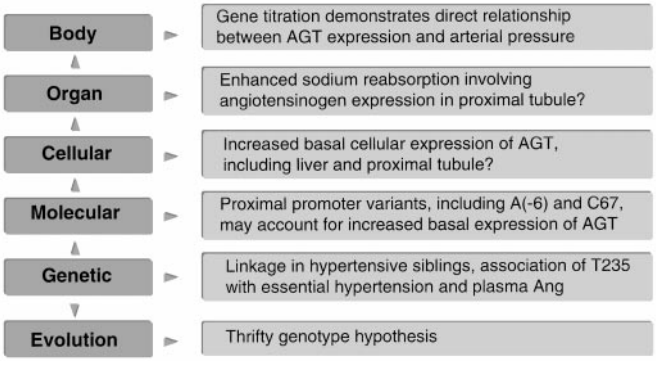

Fig. 4. The angiotensinogen (*Ang*) hypothesis. *AGT* Angiotensinogen gene

Conclusion

If identifying genetic determinants of essential hypertension was not challenging enough, understanding the physiological consequences of genetic variation in arterial pressure regulation has proven even more daunting. Although the three forays described above illustrate potentially significant advances in the delineation and the refinement of mechanistic hypotheses, they still represent work in progress, with finishing touches missing, in varying extent, to the overall picture. In each instance, a hypothesis has developed that affords the definition of future experiments, some potential advance in the line from genetic risk to clinical manifestation, and specific therapy. It is still too early to predict the extent to which each hypothesis will translate into actual or practical inferences in the hypertension field. It is also fair to acknowledge that the general reaction of various authorities to the work discussed here has ranged from genuine interest to outright suspicion or dismissal. A variety of reasons can be offered for the spread of these opinions.

To begin with, the remarkable successes of modern genetics with single Mendelian disorders may have generated undue expectations in the area of common diseases. Gene mapping and positional cloning have afforded the unambiguous identification of genes implicated in rare inherited disorders on the basis of limited but concentrated genealogical information, and gain or loss of function has commonly been deduced from direct examination of the DNA sequence. Such techniques are not likely to be effective in common diseases. A general debate over such issues has been aired in leading scientific periodicals as well as in the general press, as corporate ventures have engaged investors and citizens in large-scale population studies aimed at identifying and treating genetic determinants of common diseases. The debate about Iceland and the ambitious goals of the deCODE Corporation has crystallized scientific and public opinion, and references to friends and foes alike can be found on the Web (http://www.decode.is; http://www.mannvernd.is).

Another reason for the rapid growth of either controversy or indifference regarding hypotheses on the genetics of common diseases is that the initial inference derives from statistical tests based on designs that afford limited experimental control. Replication attempts may or may not provide additional support, for a variety of reasons that have been discussed elsewhere (Corvol et al. 1999), including population stratification, clinical heterogeneity, variation in inclusion or exclusion criteria, and limited statistical power. We take on the issues of replication and power in a separate manuscript (in preparation). The field of epidemiology abounds with examples of such issues, precisely because it is the business of statistics to derive measures of uncertainty. A statistical hypothesis does generate opportunities for statistical replication. It also affords the delineation of further studies to test refined, mechanistic hypotheses with increasing levels of specificity and experimental control. The ultimate proof of the causal link between naturally occurring

genetic variation and predisposition to common diseases may require genetic manipulations at the cellular, organ, or whole-body level. Until then, hypotheses must be appreciated for their real value. They afford the delineation of experimental tests that progressively advance our understanding of a complex phenotype. This step-by-step approach is common in the analysis of complex processes in the biological or the physical sciences, where stage-wise improvement ultimately delivers a description of the entire process in its full beauty and complexity.

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