Genetic Susceptibility to Essential Hypertension: Insight From Angiotensinogen

Jean-Marc Lalouel and Andreas Rohrwasser

Hypertension. 2007;49:597-603; originally published online January 22, 2007;
doi: 10.1161/01.HYP.0000257145.20363.9c
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563

Genetic Susceptibility to Essential Hypertension
Insight From Angiotensinogen

Jean-Marc Lalouel, Andreas Rohrwasser

Abstract—Although progress in the genetics of essential hypertension may seem disappointing, it has considerable potential in defining research directions that will ultimately translate into clinical practice. The hypothesis that genetic variation at the angiotensinogen locus impacts on individual susceptibility to develop essential hypertension has motivated a substantial body of research by us and many others. We examine how analyses of the mechanisms by which variation in angiotensinogen expression may contribute to disease susceptibility and may have arisen in human populations have progressed in recent years. Although the objective of personalized medicine is still in the future, a genetic hypothesis based on human variation can uniquely empower functional genomics approaches to reach such an ultimate goal. **(*Hypertension*. 2007;49[part 2]:597-603.)**

Key Words: genetics ■ essential hypertension ■ high blood pressure ■ angiotensinogen ■ renin

By modeling physiological systems controlling blood pressure (BP) through rigorous application of the theory of feedback control systems, Guyton[1] has established that sustained elevation of arterial pressure could be achieved by only 1 of 2 possible mechanisms: (1) general vasoconstriction including the renal arteries or (2) excess sodium retention through the kidney.

The dominant role of the kidney in BP control was remarkably confirmed by the discovery that, in all mendelian forms of hypertension or hypotension, the mutated gene products affect net renal sodium reabsorption.[2] By contrast, progress in the genetics of essential hypertension (EH) may seem disappointing. In EH, molecular variation at a number of loci increases susceptibility to disease in the context of environmental exposures through a variety of physiological mechanisms, thereby creating a complex, multivariate puzzle that presents a considerable analytical challenge.[3]

We will outline our collective experience with the angiotensinogen (AGT) hypothesis. Similar progress has been made for other genetic hypotheses, such as α-adducin or G-protein β3 subunit gene.[4,5] It follows that this article is not a review; rather, it subsumes the experience and the contribution of a group as it relates to a field of inquiry. Reference to literature in the field is far from exhaustive; to all those who have made meaningful contributions to the subject that we have failed to cite, we extend our sincere apologies.

Linkage and Association Between AGT and EH: The Development of a Genetic Hypothesis

In collaboration with Roger Williams and Pierre Corvol, we performed a genetic study focusing on the renin–angiotensin system (RAS). We chose to rely on the simple but robust approach of testing genetic linkage in affected sibling pairs, particularly attractive for diseases with complex etiology. We examined the only 3 genes of the RAS that had been cloned at the time and for which multiallelic markers were at hand. Although no linkage support was obtained for either the renin or angiotensin-converting enzyme, significant linkage was obtained in 2 independent samples of hypertensive siblings ascertained in Utah and France, respectively.[6] After the identification of common variants in the gene, significant association between the M/T (235) polymorphism and both hypertension and plasma AGT concentration was found in both samples.[6] The observation was thereafter extended to a Japanese population.[7] Increased plasma AGT in estrogenic states led us to test whether the association may extend to preeclampsia, and our data indeed supported the hypothesis.[8]

The work stimulated many attempts at replication in samples where cases were defined following diverse criteria, with few bearing on familial cases of hypertension as did our studies. We have addressed elsewhere the potential pitfalls of such replication studies.[9] Although it would seem of little relevance to estimate an attributable risk in the aggregate for a condition as heterogeneous as EH, it is reassuring that subsequent meta-analyses of this extensive literature support the conclusion of a significant statistical association between this common AGT variant and EH,[10,11] particularly when data are stratified according to family history. Evidently, significance of a genetic test did not provide functional clues about the underlying molecular mechanisms involved.

Implications of Genetic Hypotheses

Multiple organs and hundreds of regulatory pathways involving thousands of genes are likely to be involved in BP regulation. Pharmacological or genetic manipulation of any 1

Received November 1, 2006; first decision November 11, 2006; revision accepted December 22, 2006.

From the Department of Human Genetics, Eccles Institute of Human Genetics, University of Utah School of Medicine, Salt Lake City.

Correspondence to Jean-Marc Lalouel, Department of Human Genetics, Eccles Institute of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT 84112. E-mail jml@genetics.utah.edu

Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000257145.20363.9c**

gene can provide clues on the function of its product in a complex regulatory pathway and attendant consequences for BP.

Given the heterogeneity of EH and the well-documented heritability of BP, the rationale to pursue a genetic hypothesis, namely that variation at a given locus contributes to individual susceptibility to EH, is that, by addressing actual causal determinants of these inherited tendencies, it will usher diagnostic and therapeutic means that afford targeting of individual disease mechanisms. Such causal, as opposed to symptomatic, therapy is the legitimate goal of personalized medicine. The analogy that, whereas antipyretic agents can control fever and pain caused by a bacterial infection, targeted antibiotics actually treat the cause of the disease can be extended to EH. The limitation of common antihypertensive agents is that, lacking knowledge of disease mechanism in any given individual, they may leave such factors unchecked, whether or not they do control BP.

Proceeding from detection of a gene at which individual variation predisposes to EH to identification of the underlying mechanism remains a daunting task, because proof must develop through molecular, cellular, organ, and whole-body levels to ultimately establish the link between individual genetic diversity and disease.[12] Furthermore, identifying the evolutionary mechanisms that account for such genetic diversity in present-day populations of the world remains a matter of considerable interest.

As we trace progress made along the AGT hypothesis, we must acknowledge the fact that, where multiple genes are likely to be involved, practical applications will ultimately require biological integration before delineation of specific interventions. Although we are years away from such integration, the resolution of individual factors remains a necessary prerequisite.

Searching for Differences in AGT Expression in Cellular Models and in Tissues

On the Physiological Significance of Plasma AGT Concentration

Linkage and association have elevated the candidate gene status of AGT.[13,14] Whereas acute and marked changes in renin expression are essential for short-term regulation of BP, the slower, smaller, but sustained changes in AGT expression may be relevant in long-term regulation of baseline BP. A plasma concentration close to the Michaelis constant of the reaction[15] indicates that moderate changes in its plasma concentration, as observed in our genetic study,[6] could affect steady-state angiotensin formation in the circulation.

A Promoter Variant, G/A (−6), in Quasicomplete Disequilibrium With M/T (235) May Be Functionally Relevant

Our initial study could not settle whether T235 was causally involved or only served as a marker for yet ≥1 variant in linkage disequilibrium. In subsequent experiments, we could find no evidence for a functional effect when the M/T (235) transcripts and proteins were examined in vitro.[16] A possible functional role was suggested in a separate study by Gimenez-Roqueplo et al[17] through differential interaction of the M/T (235) polymorphism with Cys (232), a residue that could be important in protein folding and complex formation with other plasma proteins.

In further genetic analyses, we noted that a common variant at nucleotide −6, located between the TATA box and the transcription initiation site of AGT, was in quasicomplete disequilibrium with the M/T (235) polymorphism, with A (−6) occurring with T235, whereas G (−6) was found with M235.[16] The potential functional role of this proximal promoter variant was examined through DNA binding and in vitro transcription studies in cultured cells. These studies supported a possible impact of the variant on baseline AGT transcription, with an estimated effect of the A allele 40% to 70% higher than observed for the G allele. Although these observations supported a differential expression hypothesis, we cautioned that, "it is clearly not possible to directly extend the results of transfection experiments done with truncated AGT promoters in cultured cells to the function of the intact gene at the level of the whole organism." Another polymorphism, T/C (67); denoted "68" in Reference 18 in complete association with polymorphisms at −6 and codon 235, was present in the constructs used in our in vitro tests and, therefore, could also account in part for our observations.

Allelic Differences in AGT Expression at the Level of the Tissues

The quasicomplete linkage disequilibrium between these 2 polymorphisms suggested that the hypothesis of differential mRNA expression could be directly addressed using the M/T (235) polymorphism to tag transcripts. Although this could be pursued in human liver tissues, we had no access to such samples. Pursuing our hypothesis of a relationship between AGT polymorphism and preeclampsia with Morgan et al,[19] however, we inspected placental samples obtained from first-trimester elective abortions. First, we established that AGT was expressed in decidual tissues, where it was restricted to smooth muscle cells of spiral arteries. Maternal decidua from 39 individuals heterozygous for the M/T (235) polymorphism were used to quantitate mRNA carrying the M235 and the T235 alleles by each of 2 distinct methods, an allele-specific ligation assay and a single nucleotide primer extension assay. In either assay, the T235 transcript was significantly higher than its M235 counterpart. The difference observed was ≈2-fold, but the quantitative aspect of this ratio cannot be interpreted directly in view of the nonlinearity of the reactions involved. Whether similar differences can be observed in other AGT-expressing tissues remains unknown.

Direct Demonstrations in Animal Models

In early transgenic experiments, overexpression of rat AGT in the mouse led to fulminant hypertension.[20,21] In subsequent work, the well-documented species specificity of the renin–AGT reaction was exploited by overexpressing both human renin and human AGT in mouse.[22,23] Again, in all instances, very severe hypertension was observed.

In all of these models, clamping both human renin and human AGT at high, unregulated levels produced a form of hypertension somewhat akin to that produced by chronic angiotension II (Ang II) infusion at marked pressor levels. Indeed, in mice doubly transgenic for human renin and

human AGT, plasma renin activity was increased 10-fold, whereas plasma human AGT was 150-fold higher than in human plasma.[24] These studies provided remarkable proof of principle of the significance of the RAS in BP regulation.

The generation of animals with graded, modest changes in AGT expression in the physiological range postulated in humans was made possible by the original development of the gene titration approach.[25,26] Through the combination of classical and a special form of gap repair gene targeting, Smithies and Kim[25] generated and characterized animals with mouse AGT copy number varying from 1 to 4. Three-copy animals exhibited a 24% increase in plasma AGT concentration and an 8-mm Hg increase in mean arterial pressure as measured directly by indwelling catheters[26] when compared with 2-copy animals.

Systemic Versus Tissue Systems: The Possible Participation of a Tubular RAS

The overlap of systemic and tissue RAS through filtration, transport, or diffusion of soluble components to extracellular space and tissues renders challenging any apportioning of their relative contributions to BP regulation. The well-recognized role of intrarenally derived Ang II in BP regulation and renal function[27,28] suggested that AGT differential expression in the proximal tubule (PT) may contribute to an AGT-mediated susceptibility to hypertension. To address this issue, Ding et al[29] and Davisson et al[30] generated doubly transgenic mice overexpressing human renin under its own promoter and human AGT under the testosterone-dependent kidney androgen-regulated protein promoter, restricting human AGT expression to PT. In this model, mean arterial pressure was increased 30 to 40 mm Hg in male mice, and a similar increase was induced in female mice upon testosterone administration. No human AGT could be detected in the circulation, and circulating mouse AGT and Ang II were in the reference range, whereas human AGT was easily detected in urine. This model suggested that increased expression of AGT in PT could lead to enhanced local production of Ang II and that this mechanism could lead to hypertension without direct participation of the systemic RAS. Taken together, these various animal models were consistent with the long-held view that systemic and intrarenal RAS could contribute to chronic elevation in arterial pressure through distinct mechanisms.

Delineation of a Tubular RAS Along the Entire Nephron

Intrarenal RAS and Tubular RAS

A variety of experiments document the participation of an intrarenal RAS in sodium reabsorption and BP regulation.[28,31] The presence of angiotensin-converting enzyme and angiotensin type I receptors along the nephron has been well established,[32,33] and AGT was long known to be expressed in PT.[34] The leading evidence for an active tubular RAS in PT was the observation of Ang II at a concentration ≈50-fold higher in luminal fluid than in plasma[35,36] and that Ang II in this segment enhances sodium reabsorption through stimulation of the sodium/hydrogen exchanger.[37]

Less evident is the actual mechanism of formation of Ang II in PT, as well as in distal segments of the nephron.[31] Ang I or Ang II could be formed intracellularly and secreted in tubular lumen, or alternately AGT could be secreted and cleaved in tubular fluid. The former would require the action of intracellular renin or another enzyme capable of releasing angiotensins. The latter would require the presence of renin in the lumen of PT, whether locally generated or of filtered origin.

AGT Expression in PT Cells and Secretion in Tubular Fluid

AGT expression in PT has long been recognized.[34] Wang et al,[38] finding an androgen-dependent correlation between renal AGT mRNA and excretion of the protein in urine with no parallel change in plasma AGT, provided initial support for the intrarenal origin of urinary AGT. We confirmed urinary AGT excretion and provided evidence of its apical secretion by PT monolayers in culture.[39]Appearance in urine but not in plasma of human AGT overexpressed in mouse PT provided further evidence of luminal secretion of AGT in PT.[30] Kobori et al[40–42] have demonstrated significant increases of both intrarenal AGT mRNA and protein on sustained infusion of Ang II. They also demonstrated AGT excretion in urine in this model.[42,43]

The Origin of Renin in PT

Some experimental data suggest renin expression in PT.[44,45] A more evident source of renin in PT lumen is filtration of circulating renin, because the kidney accounts for the rapid clearance of renin from the circulation. Most filtered renin is reabsorbed along the PT.[46,47] Although local production of renin may serve local intracrine or autocrine functions, filtered renin may be a more abundant source of the protein in PT lumen under most physiological conditions. Rather than just catabolism, glomerular filtration of renin may represent active delivery to act on PT AGT in tubular fluid.[39]

Expression of Renin in the Connecting Tubule and Collecting Duct

Transit of some intact AGT through the nephron led us to search for renin expression at a distal tubular site. Sodium restriction induced renin immunostaining in cells of open tubular segments identified as connecting tubule (CNT) cells based on topographical and morphological arguments, mutual exclusion from H^+-ATPase,[39] and colocalization with tissue kallikrein expression.[48] Renin immunostaining, occasionally reported in tubular segments of mouse kidney,[49,50] was either dismissed as an artifact or interpreted as nonspecific reuptake of filtered renin. We have provided conclusive evidence of CNT synthesis through detection of renin mRNA by both in situ RT-PCR and by RT-PCR from microdissected nephron segments.[39]

Renin expression in distal segments of the nephron under physiological conditions received further support when similar observations were reported in pathological[51,52] and pharmacological models.[53] Although renin activation may be restricted to CNT under physiological conditions,[39] recruitment of collecting duct occurs in response to more extreme stimuli, such as sustained Ang II infusion.[53] A similar observation has been noted for epithelial sodium channel expression, when *Hoxb7*-directed knockout of epithelial sodium channel in collecting duct revealed that CNT was sufficient to handle distal sodium

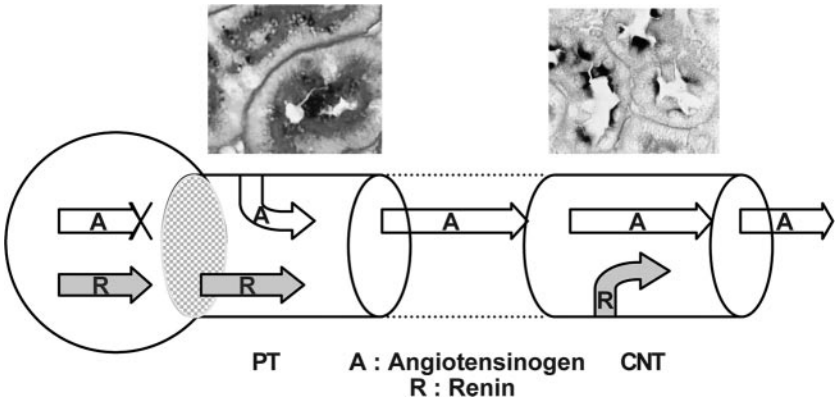

Figure 1. Participation of renin and AGT to a paracrine tubular RAS along the entire nephron.

reabsorption in response to dietary sodium changes.[54,55] Renin-driven Cre recombinase activation of a reporter gene during development did reveal that renin was transiently expressed in the renal collecting duct during kidney development.[56]

Proposal: A Paracrine Tubular RAS Integrating Proximal and Distal Functions

Overlap between systemic and intrarenal RAS has long been recognized and is often described in terms of the ensuing compartmentalization of Ang II in the kidney and its implication for renal sodium handling, BP regulation, and treatment of hypertension.[57] Our work led us to propose that a paracrine tubular RAS that includes as soluble precursors filtered renin of systemic origin, PT AGT, and renin in CNT could play a significant role in the coordinated regulation of sodium reabsorption between proximal and distal sites along the nephron[39] (Figure 1). The role of the proximal component of this system in bulk sodium transport is well recognized.[28,37] CNT renin expression and its action on AGT of PT origin could prove critical in volume-depleted states or in situations of relative or absolute sodium deficit.

A role for Ang II in the regulation of sodium reabsorption at distal sites of the nephron has long been argued.[27,58] Evidence supporting luminal effects of A-II in epithelial sodium channel function in distal nephron segments[59] has now received considerable support.[60,61]

Plasma and Urinary AGT: Origin, Physiological Significance, and Potential Clinical Use

The increased focus borne onto plasma and intrarenal AGT raises the issues described here. What is the origin of plasma AGT? Is it primarily of hepatic origin? Does its correlation with BP, long noted by Walker et al,[62] supported by the joint association with EH and the M/T (235) polymorphism, and again observed in the gene titration model described previously,[25,26] reflect a direct, causal effect? Does urinary AGT (U-AGT) derive from plasma or from PT? Does urinary excretion of AGT reflect its tubular production? What is its potential clinical use?

Although transgenic overexpression of AGT in adipose tissue leads to increased plasma AGT and increased BP,[63] evidence indicates that, under normal physiological conditions, plasma AGT is primarily of hepatic origin.[64] The correlation between increased adiposity and plasma AGT warrants further analysis of this issue. Increased AGT expression with AGT gene copy number leading to both increased plasma AGT and BP[25,26] does not imply causal dependency between these 2 phenotypes.

AGT in urine can be of systemic origin in pathological models that affect the integrity of the glomerular membrane. There is substantial evidence, however, that in most physiological states, U-AGT is primarily of PT origin. This was suggested by the work of Wang et al.[38] In the Ang II–infused model of hypertension in rat, Kobori et al[42,65] found that both renal AGT mRNA and protein, as well as U-AGT, were markedly increased, with no parallel effect on plasma AGT. When human AGT was infused intravenously, it was not detected in urine.[65] In transgenic mice with human AGT expression restricted to PT,[30] the protein was detected in urine. Lastly, U-AGT excretion did not parallel plasma AGT in response to step changes in dietary sodium in mice.[66]

Although U-AGT excretion does reflect intrarenal production in the Ang II infusion model of hypertension,[40] under physiological conditions, this relationship may be obscured by the extent of tubular reabsorption of AGT so that U-AGT excretion may reflect net water and sodium reabsorption in this segment and, therefore, PT function and dietary sodium more than its PT production rate.[66]

To evaluate the potential clinical use of U-AGT in EH, we analyzed this parameter in a sample of consecutively ascertained French patients.[67] To define more homogeneous subsets, patients were classified on the basis of their renin and aldosterone status under their usual sodium intake. We partitioned patients simply on the basis of medians of upright plasma active renin concentration and plasma aldosterone. Two groups were delineated: patients with renin and aldosterone below medians and other patients. The most striking feature was the observation of a strong (≈0.50) correlation between U-AGT and both systolic and diastolic BP obtained by 24-hour monitoring in female patients with renin and aldosterone below medians but in no other groups. Although these data must be considered preliminary, they suggest that there is merit to consider the potential use of U-AGT in further studies where dietary sodium would be controlled.

Evolutionary Implications

If common genetic variants account for susceptibility to common disease, the reason for their high frequency in some

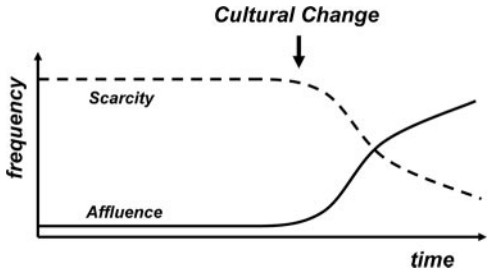

Figure 2. The lag of genetic over cultural change.

populations remains a "first-class mystery."[68] The issue can be discussed without knowing which of the many AGT common variants account for increased or reduced susceptibility to EH. This is because, on resequencing 736 entire AGT genes in multiple samples from Africa, Asia, and Europe, we not only confirmed the quasiabsolute association between variants at −6 and at codon 235 but also found that a unique G(−6)-M235 haplotype differed from multiple A(−6)-T235 haplotypes at 13 nucleotide positions (including nucleotide 67).[69] The presence of A and T at −6 and 235 in all of the primates resequenced[16] strongly suggests that A(−6)-T235 represents ancestral haplotypes, whereas G(−6)-M235 tags a more recently derived haplotype or neomorph. Restricted distribution of alleles at a multiallelic microsatellite[70] supports that inference. We will use codon 235 to refer to the derived M235 haplotype and the ancestral T235 haplotypes.

The M235-derived haplotype exhibited marked variation in frequency among world populations.[16,69] Rare in Central Africa (0.02), it is low in Asia (0.15 to 0.25), intermediate in the Middle East and Eastern Europe (≈0.50), and highest in populations of Northern European descent sampled in Utah (0.70). A number of evolutionary tests were applied to estimate the emergence time of the M235 haplotype, leading to an estimate as recent as 22 000 to 44 000 years.[69]

The rapid increase in frequency of this derived haplotype could have resulted either from a marked bottleneck effect or from natural selection. Evolutionary analyses do suggest that this increase in frequency in Asian and European populations may have indeed resulted from a selective sweep,[69] supporting the hypothesis that M235 may have increased recently in populations of Asia and Europe in response to selective forces favoring this haplotype over ancestral T235 haplotypes. This would fit the thrifty genotype hypothesis proposed by Neel in 1962[68] His hypothesis postulates that ancestral haplotype(s) (tagged here by T235 at AGT), advantageous when faced with scarcity for a substance essential for life, could become deleterious when cultural change renders that substance abundant enough to allow excess consumption (Figure 2). For an AGT-mediated susceptibility to hypertension, the likely substance would be sodium. There is much evidence of long-standing primate and human evolution in Africa under conditions of extreme sodium scarcity, with greater abundance and excess consumption as humans spread out of Africa.[71]

Under Neel's[68] hypothesis, the genetics of common disease would represent the lag of genetic over cultural change. One may wonder how the selective forces at play may have operated, given that EH became manifest and affected lifespan after the reproductive period for much of the evolutionary history of humans. In follow-up to the report of a significant association between the M/T (235) and preeclampsia in primigravida,[8] we have shown that AGT was expressed in spiral arteries in first-trimester placenta, with T235 expressed at a higher level than M235 in heterozygous specimens.[19] Consequently, susceptibility to preeclampsia mediated by T235 may have and continues to provide the selective force for the rise of M235 and the fall of T235 in human populations, in quantitative terms consistent with mathematical expectations.[72]

If the homogeneous M235-derived haplotype favored sodium tolerance, it remains conceivable that the multiple ancestral T235 (and -A[−6]) haplotypes differ in their tendency to promote sodium sensitivity and, therefore, AGT-mediated susceptibility to hypertension. This issue has been addressed and reviewed by Kumar et al.[73] Perhaps not too surprisingly, these reports strongly suggest that variability at the AGT locus mediates susceptibility to disease in a more complex manner than initially anticipated.

Perspectives

The hypothesis of AGT variation as a genetic susceptibility to EH has motivated considerable research to further our understanding of the evolutionary history and the mechanism by which genetic variation at this locus can affect sodium handling and BP regulation. This work and similar efforts directed toward the genetics of EH highlight future perspectives to reach the goals of understanding individual susceptibility to disease so that it translates into clinical practice. Past and current experience, combined with emerging technologies, may ultimately usher personalized medicine and individual therapy.

At the level of the individual, the hypertension phenotype has proven much too vague for genetic investigations, a limitation not alleviated by the substitution of BP. This is very well summarized by Ferrari and Bianchi,[74] who emphasize the complexity introduced by variation attributable not only to underlying mechanisms but also to stages of disease, treatment, and other confounders. What are needed are careful investigations of physiological systemic and renal responses to controlled stimuli (such as step changes in sodium intake, plasma volume, or response to specific antihypertensive agents) in select subjects at genetic risk of disease, exploiting the time dependence and the multivariate aspects of such responses.

Likewise, animal models are needed to investigate organ- and tissue-specific responses to genetic perturbations in the physiological range, because the deviations and the return to equilibrium of complex systems after modest versus drastic perturbations are not likely to be the same. Indeed, functional analysis of equilibrium in systems involving multiple parameters by applying discrete changes in ≥1 parameter may shift to new equilibria in a complex universe that contains many peaks and valleys. Not only should the stimuli considered to probe the response of the system be physiologically relevant in scale and in direction, but responses to any stimulus need to be investigated at the level of an entire system. This "systems biology" approach is now receiving considerable attention, and functional genomic tools are allowing the investigator to develop this broader analytical

perspective. Some investigators have taken a head start in this direction, as did Cowley[75] in a global genomics and homeostasis approach, who pointed out the analogy with Guyton's models in integrative physiology. On the smaller scale of investigating response to a single, modest genetic perturbation, Smithies[76] again was 1 of the earlier advocates of a systems approach with 2 distinct but complementary contributions. First, he attempted to model the response of the RAS to genetic perturbations of its individual components in mathematical terms, providing a unique outlook, as well as specific and testable predictions. In another seminal contribution, he investigated the response, at the transcriptional level, not only of components of the RAS, but also of key components of regulatory systems likely to be affected by specific alterations in AGT copy number.[77]

Given the obvious complexity of physiological regulation at the molecular level and the relevance of homeostatic regulation for common disease, we can no longer simply probe pairwise relationships in complex networks involving hundreds of components. Phenotypic characterization using functional genomics tools becomes an inescapable necessity.[78] Such investigations are best justified when the perturbation applied to a system rests in genetic observations initially made in humans. We think that great advances will be achieved in the field of hypertension research when such tools are applied with a sound hypothesis and a clear rationale.

Acknowledgments

This article is dedicated to the memory of Roger R. Williams. We collectively refer to our work and that of our many collaborators over the years in a collegial fashion, citing the corresponding literature, and as such gratefully acknowledge their invaluable contributions.

Sources of Funding

This work was supported by the Howard Hughes Medical Institute and National Institutes of Health grants HL45325, HL52501, and HL55000.

Disclosures

None.

References

1. Guyton AC. *Arterial Pressure and Hypertension.* Philadelphia, PA: W.B. Saunders; 1980.
2. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell*. 2001;104:545–556.
3. Lalouel JM, Rohrwasser A, Terreros D, Morgan T, Ward K. Angiotensinogen in essential hypertension: from genetics to nephrology. *J Am Soc Nephrol*. 2001;12:606–615.
4. Bianchi G. Genetic variations of tubular sodium reabsorption leading to "primary" hypertension: from gene polymorphism to clinical symptoms. *Am J Physiol Regul Integr Comp Physiol*. 2005;289:R1536–R1549.
5. Siffert W. G protein polymorphisms in hypertension, atherosclerosis, and diabetes. *Annu Rev Med*. 2005;56:17–28.
6. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel JM, Corvol P. Molecular basis of human hypertension: role of angiotensinogen. *Cell*. 1992;71:169–180.
7. Hata A, Namikawa C, Sasaki M, Sato K, Nakamura T, Tamura K, Lalouel JM. Angiotensinogen as a risk factor for essential hypertension in Japan. *J Clin Invest*. 1994;93:1285–1287.
8. Ward K, Hata A, Jeunemaitre X, Helin C, Nelson L, Namikawa C, Farrington PF, Ogasawara M, Suzumori K, Tomoda S, Berrebi S, Sasaki M, Corvol P, Lifton RP, Lalouel JM. A molecular variant of angiotensinogen associated with preeclampsia. *Nat Genet*. 1993;4:59–61.
9. Lalouel JM, Rohrwasser A. Power and replication in case-control studies. *Am J Hypertens*. 2002;15:201–205.
10. Kunz R, Kreutz R, Beige J, Distler A, Sharma AM. Association between the angiotensinogen 235T-variant and essential hypertension in whites: a systematic review and methodological appraisal. *Hypertension*. 1997;30:1331–1337.
11. Staessen JA, Kuznetsova T, Wang JG, Emelianov D, Vlietinck R, Fagard R. M235T angiotensinogen gene polymorphism and cardiovascular renal risk. *J Hypertens*. 1999;17:9–17.
12. Lalouel JM. From genetics to mechanism of disease liability. In: Rao DC, Province MA, eds. *Genetic Dissection of Complex Traits.* St Louis, MO: Academic Press; 2001:517–533.
13. Gordon DB. The role of renin substrate in hypertension. *Hypertension*. 1983;5:353–362.
14. Menard J, el Amrani AI, Savoie F, Bouhnik J. Angiotensinogen: an attractive and underrated participant in hypertension and inflammation [letter; comment]. *Hypertension*. 1991;18:705–707.
15. Gould AB, Green D. Kinetics of the human renin and human substrate reaction. *Cardiovasc Res*. 1971;5:86–89.
16. Inoue I, Nakajima T, Williams CS, Quackenbush J, Puryear R, Powers M, Cheng T, Ludwig EH, Sharma AM, Hata A, Jeunemaitre X, Lalouel JM. A nucleotide substitution in the promoter of human angiotensinogen is associated with essential hypertension and affects basal transcription in vitro. *J Clin Invest*. 1997;99:1786–1797.
17. Gimenez-Roqueplo AP, Celerier J, Schmid G, Corvol P, Jeunemaitre X. Role of cysteine residues in human angiotensinogen. Cys232 is required for angiotensinogen-pro major basic protein complex formation. *J Biol Chem*. 1998;273:34480–34487.
18. Ishigami T, Tamura K, Fujita T, Kobayashi I, Hibi K, Kihara M, Toya Y, Ochiai H, Umemura S. Angiotensinogen gene polymorphism near transcription start site and blood pressure: role of a T-to-C transition at intron I. *Hypertension*. 1999;34:430–434.
19. Morgan T, Craven C, Nelson L, Lalouel JM, Ward K. Angiotensinogen T235 expression is elevated in decidual spiral arteries. *J Clin Invest*. 1997;100:1406–1415.
20. Ohkubo H, Kawakami H, Kakehi Y, Takumi T, Arai H, Yokota Y, Iwai M, Tanabe Y, Masu M, Hata J, Iwao H, Okamoto H, Yokoyama M, Nomura T, Katsuki M, Nakanishi S. Generation of transgenic mice with elevated blood pressure by introduction of the rat renin and angiotensinogen genes. *Proc Natl Acad Sci U S A*. 1990;87:5153–5157.
21. Kimura S, Mullins JJ, Bunnemann B, Metzger R, Hilgenfeldt U, Zimmermann F, Jacob H, Fuxe K, Ganten D, Kaling M. High blood pressure in transgenic mice carrying the rat angiotensinogen gene. *EMBO J*. 1992;11:821–827.
22. Uehara S, Mino N, Tsuchida M, Nishikibe M, Fukamizu A, Murakami K, Ikemoto F Development of polyuria in Tsukuba hypertensive mice carrying human renin and angiotensinogen genes. *Clin Exp Pharmacol Physiol.* 1995;22(suppl 1):S12–S14.
23. Merrill DC, Thompson MW, Carney CL, Granwehr BP, Schlager G, Robillard JE, Sigmund CD. Chronic hypertension and altered baroreflex responses in transgenic mice containing the human renin and human angiotensinogen genes. *J Clin Invest*. 1996;97:1047–1055.
24. Yang G, Merrill DC, Thompson MW, Robillard JE, Sigmund CD. Functional expression of the human angiotensinogen gene in transgenic mice. *J Biol Chem*. 1994;269:32497–32502.
25. Smithies O, Kim HS. Targeted gene duplication and disruption for analyzing quantitative genetic traits in mice. *Proc Natl Acad Sci U S A*. 1994;91:3612–3615.
26. Kim HS, Krege JH, Kluckman KD, Hagaman JR, Hodgin JB, Best CF, Jennette JC, Coffman TM, Maeda N, Smithies O. Genetic control of blood pressure and the angiotensinogen locus. *Proc Natl Acad Sci U S A*. 1995;92:2735–2739.
27. Hall J, Brands M. Intrarenal and circulating angiotensin II and renal function. In: Robertson J, Nicholls M, eds. *The Renin-Angiotensin System.* Vol 1. New York, NY: Gower Medical Publishing; 1993;26:21–26.43.
28. Navar LG, Imig JD, Zou L, Wang CT. Intrarenal production of angiotensin II. *Semin Nephrol*. 1997;17:412–422.
29. Ding Y, Davisson RL, Hardy DO, Zhu LJ, Merrill DC, Catterall JF, Sigmund CD. The kidney androgen-regulated protein promoter confers renal proximal tubule cell-specific and highly androgen-responsive expression on the human angiotensinogen gene in transgenic mice. *J Biol Chem*. 1997;272:28142–28148.
30. Davisson RL, Ding Y, Stec DE, Catterall JF, Sigmund CD. Novel mechanism of hypertension revealed by cell-specific targeting of human angiotensinogen in transgenic mice. *Physiol Genomics*. 1999;1:3–9.

31. Navar LG, Harrison-Bernard LM, Wang CT, Cervenka L, Mitchell KD. Concentrations and actions of intraluminal angiotensin II. *J Am Soc Nephrol*. 1999;10(suppl 11):S189–S195.
32. Casarini DE, Boim MA, Stella RC, Krieger-Azzolini MH, Krieger JE, Schor N. Angiotensin I-converting enzyme activity in tubular fluid along the rat nephron. *Am J Physiol*. 1997;272:F405–F409.
33. Harrison-Bernard LM, Navar LG, Ho MM, Vinson GP, el-Dahr SS. Immunohistochemical localization of ANG II AT1 receptor in adult rat kidney using a monoclonal antibody. *Am J Physiol*. 1997;273: F170–F177.
34. Ingelfinger JR, Pratt RE, Ellison K, Dzau VJ. Sodium regulation of angiotensinogen mRNA expression in rat kidney cortex and medulla. *J Clin Invest*. 1986;78:1311–1315.
35. Seikaly MG, Arant BSJ, Seney FDJ. Endogenous angiotensin concentrations in specific intrarenal fluid compartments of the rat. *J Clin Invest*. 1990;86:1352–1357.
36. Braam B, Mitchell KD, Fox J, Navar LG. Proximal tubular secretion of angiotensin II in rats. *Am J Physiol*. 1993;264:F891–F898.
37. Quan A, Baum M. Regulation of proximal tubule transport by angiotensin II. *Semin Nephrol*. 1997;17:423–430.
38. Wang E, Yayama K, Takano M, Okamoto H. Sexual dimorphism of urine angiotensinogen excretion in the rat. *Jpn J Pharmacol*. 1994;64:243–250.
39. Rohrwasser A, Morgan T, Dillon HF, Zhao L, Callaway CW, Hillas E, Zhang S, Cheng T, Inagami T, Ward K, Terreros DA, Lalouel JM. Elements of a paracrine tubular renin-angiotensin system along the entire nephron. *Hypertension*. 1999;34:1265–1274.
40. Kobori H, Harrison-Bernard LM, Navar LG. Enhancement of angiotensinogen expression in angiotensin II-dependent hypertension. *Hypertension*. 2001;37:1329–1335.
41. Kobori H, Harrison-Bernard LM, Navar LG. Expression of angiotensinogen mRNA and protein in angiotensin II-dependent hypertension. *J Am Soc Nephrol*. 2001;12:431–439.
42. Kobori H, Harrison-Bernard LM, Navar LG. Urinary excretion of angiotensinogen reflects intrarenal angiotensinogen production. *Kidney Int*. 2002;61:579–585.
43. Kobori H, Nishiyama A, Abe Y, Navar LG. Enhancement of intrarenal angiotensinogen in Dahl salt-sensitive rats on high salt diet. *Hypertension*. 2003;41:592–597.
44. Moe OW, Alpern RJ, Henrich WL. The renal proximal tubule renin-angiotensin system. *Semin Nephrol*. 1993;13:552–557.
45. Chen M, Harris MP, Rose D, Smart A, He XR, Kretzler M, Briggs JP, Schnermann J. Renin and renin mRNA in proximal tubules of the rat kidney. *J Clin Invest*. 1994;94:237–243.
46. Leyssac PP. A micropuncture study of glomerular filtration and tubular reabsorption of endogenous renin in the rat. *Renal Physiol Basel*. 1978; 1:181–188.
47. Nielsen AH, Hermann KL, Mazanti I, Poulsen K. Urinary excretion of inactive renin during blockade of the renal tubular protein reabsorption with lysine. *J Hypertens*. 1989;7:77–82.
48. Rohrwasser A, Ishigami T, Gociman B, Lantelme P, Morgan T, Cheng T, Hillas E, Zhang S, Ward K, Bloch-Faure M, Meneton P, Lalouel JM. Renin and kallikrein in connecting tubule of mouse. *Kidney Int*. 2003; 64:2155–2162.
49. Celio MR, Inagami T. Renin in the human kidney. Immunohistochemical localization. *Histochemistry*. 1981;72:1–10.
50. Taugner R, Hackenthal E, Inagami T, Nobiling R, Poulsen K. Vascular and tubular renin in the kidneys of mice. *Histochemistry*. 1982;75: 473–484.
51. Gilbert RE, Wu LL, Kelly DJ, Cox A, Wilkinson-Berka JL, Johnston CI, Cooper ME. Pathological expression of renin and angiotensin II in the renal tubule after subtotal nephrectomy. Implications for the pathogenesis of tubulointerstitial fibrosis. *Am J Pathol*. 1999;155:429–440.
52. Torres VE, Donovan KA, Scicli G, Holley KE, Thibodeau SN, Carretero OA, Inagami T, McAteer JA, Johnson CM. Synthesis of renin by tubulocystic epithelium in autosomal-dominant polycystic kidney disease. *Kidney Int*. 1992;42:364–373.
53. Prieto-Carrasquero MC, Harrison-Bernard LM, Kobori H, Ozawa Y, Hering-Smith KS, Hamm LL, Navar LG. Enhancement of collecting duct renin in angiotensin II-dependent hypertensive rats. *Hypertension.* 2004; 44:223–229.
54. Rubera I, Loffing J, Palmer LG, Frindt G, Fowler-Jaeger N, Sauter D, Carroll T, McMahon A, Hummler E, Rossier BC. Collecting duct-specific gene inactivation of alphaENaC in the mouse kidney does not impair sodium and potassium balance. *J Clin Invest*. 2003;112:554–565.
55. Meneton P, Loffing J, Warnock DG. Sodium and potassium handling by the aldosterone-sensitive distal nephron: the pivotal role of the distal and connecting tubule. *Am J Physiol Renal Physiol*. 2004;287:F593–F601.
56. Castrop H, Oppermann M, Weiss Y, Huang Y, Mizel D, Lu H, Germain S, Schweda F, Theilig F, Bachmann S, Briggs J, Kurtz A, Schnermann J. Reporter gene recombination in juxtaglomerular granular and collecting duct cells by human renin promoter-Cre recombinase transgene. *Physiol Genomics*. 2006;25:277–285.
57. Siragy HM. Angiotensin II compartmentalization within the kidney: effects of salt diet and blood pressure alterations. *Curr Opin Nephrol Hypertens*. 2006;15:50–53.
58. Hall JE, Brands MW, Henegar JR. Angiotensin II and long-term arterial pressure regulation: the overriding dominance of the kidney. *J Am Soc Nephrol.* 1999;10(suppl 12):S258–S265.
59. Wang T, Giebisch G. Effects of angiotensin II on electrolyte transport in the early and late distal tubule in rat kidney. *Am J Physiol*. 1996;271: F143–F149.
60. Peti-Peterdi J, Warnock DG, Bell PD. Angiotensin II directly stimulates ENaC activity in the cortical collecting duct via AT(1) receptors. *J Am Soc Nephrol*. 2002;13:1131–1135.
61. Komlosi P, Fuson AL, Fintha A, Peti-Peterdi J, Rosivall L, Warnock DG, Bell PD. Angiotensin I conversion to angiotensin II stimulates cortical collecting duct sodium transport. *Hypertension*. 2003;42:195–199.
62. Walker WG, Saito H, Whelton PK, Russell RP, Hermann JS. An association between the renin angiotensin system, blood pressure and potassium intake. *Trans Am Clin Climatol Assoc*. 1979;91:107–114.
63. Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, Quignard-Boulange A, Negrel R, Ailhaud G, Seydoux J, Meneton P, Teboul M. Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J*. 2001;15:2727–2729.
64. Stec DE, Keen HL, Sigmund CD. Lower blood pressure in floxed angiotensinogen mice after adenoviral delivery of Cre-recombinase. *Hypertension*. 2002;39:629–633.
65. Kobori H, Nishiyama A, Harrison-Bernard LM, Navar LG. Urinary angiotensinogen as an indicator of intrarenal Angiotensin status in hypertension. *Hypertension*. 2003;41:42–49.
66. Lantelme P, Rohrwasser A, Gociman B, Hillas E, Cheng T, Petty G, Thomas J, Xiao S, Ishigami T, Herrmann T, Terreros DA, Ward K, Laloulel JM. Effects of dietary sodium and genetic background on angiotensinogen and renin in mouse. *Hypertension*. 2002;39:1007–1014.
67. Lantelme P, Rohrwasser A, Vincent M, Cheng T, Gardier S, Legedz L, Bricca G, Laloulel JM, Milon H. Significance of urinary angiotensinogen in essential hypertension as a function of plasma renin and aldosterone status. *J Hypertens*. 2005;23:785–792.
68. Neel JV. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am J Hum Genet*. 1962;14:353–362.
69. Nakajima T, Wooding S, Sakagami T, Emi M, Tokunaga K, Tamiya G, Ishigami T, Umemura S, Munkhbat B, Jin F, Guan-Jun J, Hayasaka I, Ishida T, Saitou N, Pavelka K, Laloulel JM, Jorde LB, Inoue I. Natural selection and population history in the human angiotensinogen gene (AGT): 736 complete AGT sequences in chromosomes from around the world. *Am J Hum Genet*. 2004;74:898–916.
70. Jeunemaitre X, Inoue I, Williams C, Charru A, Tichet J, Powers M, Sharma AM, Gimenez-Roqueplo AP, Hata A, Corvol P, Laloulel JM. Haplotypes of angiotensinogen in essential hypertension. *Am J Hum Genet*. 1997;60:1448–1460.
71. Denton D. *The Hunger for Salt. An Anthropological, Physiological, and Medical Analysis.* Berlin, Germany: Springer Verlag; 1984.
72. Laloulel JM. *Concentrations Locales D'affections Hereditaires Rares.* Paris, France: L'expansion Scientific Francaise; 1970.
73. Kumar A, Li Y, Patil S, Jain S. A haplotype of the angiotensinogen gene is associated with hypertension in African Americans. *Clin Exp Pharmacol Physiol.* 2005;32:495–502.
74. Ferrari P, Bianchi G. Genetic mapping and tailored antihypertensive therapy. *Cardiovasc Drugs Ther*. 2000;14:387–395.
75. Cowley AW Jr. Genomics and homeostasis. *Am J Physiol Regul Integr Comp Physiol.* 2003;284:R611–R627.
76. Smithies O. Theodore Cooper Memorial Lecture. A mouse view of hypertension. *Hypertension*. 1997;30:1318–1324.
77. Kim HS, Lee G, John SW, Maeda N, Smithies O. Molecular phenotyping for analyzing subtle genetic effects in mice: application to an angiotensinogen gene titration. *Proc Natl Acad Sci U S A*. 2002;99:4602–4607.
78. Cowley AW Jr. The genetic dissection of essential hypertension. *Nat Rev Genet.* 2006;7:829–840.