

Molecular genetics of human hypertension

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The year 1999 saw considerable activity in the area of hypertension-related molecular genetics. Several new monogenic hypertensive disorders, as well as a monogenic form of hypotension, were elucidated. Molecular genetics has made significant inroads in explaining basic mechanisms of magnesium homeostasis. Linkage strategies have been applied in family studies, sib-pair analyses, and twin studies. More stringent criteria for association studies have been formulated. The 11 β -hydroxysteroid dehydrogenase gene, the prostacyclin synthase gene, genes coding for variants in G proteins, and adrenergic receptor genes have received particular attention. On the horizon are better phenotyped patient and subject collectives, expanded genotyping with the availability of a 300 000 genome-wide single-nucleotide polymorphism map, multigenic studies in the form of metabolic control analyses, and new bioinformatic strategies including neural networks. *Curr Opin Nephrol Hypertens* 9:259–266. © 2000 Lippincott Williams & Wilkins.

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Abbreviations

ACE	angiotensin converting enzyme
ENaC	epithelial sodium channel
NOS	nitric oxide synthase
PPARγ	proliferator-activated receptor gamma
QTL	quantitative trait locus

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Introduction

Just over a year ago, when I was asked to review the molecular genetics of human hypertension, I presented the somewhat pessimistic view that much hype had accompanied some hope in this area of research. The promise that rapid progress in this area would be made, that new diagnostic procedures would fashion therapies, or that new classes of compounds would quickly be developed, was exaggerated, in my opinion [1]. The editors asked me to assess what progress has been made in the 12 months since that review was published. New data have appeared at a lively pace and the enthusiasm of investigator has evidently not been dampened. However, that new, more efficient methods of genetic analysis will yield clinically meaningful information remains a hope, not a promise [2]. Although coverage of so much active research would be beyond the bounds of this review, I will attempt to highlight progress in monogenic hypertensive or blood pressure–regulatory disorders followed by the complex genetic regulation of blood pressure. I will restrict the review to human hypertension unless certain animal investigations have specific relevance or address particularly important issues.

Monogenic disease

Monogenic forms of hypertension remain the major success stories. The most exciting findings have not yet been formally published and are only available in abstract form. At this year's Council for High Blood Pressure Research meeting, the Lifton Group (D. S. Geller, personal communication, 1999) presented a new Mendelian form of hypertension caused by an activating mutation in the mineralocorticoid receptor. These investigators screened for mutations in the mineralocorticoid receptor in seven unrelated patients referred for possible monogenic hypertension by the single-strand conformation polymorphism technique. One patient had a heterozygous mutation at codon 810 in the mineralocorticoid receptor gene, resulting in a substitution of leucine for serine, a residue which lies in the hormone-binding domain. The patient's kindred were carefully examined: the index case had severe hypertension, as did four relatives; four other relatives had no hypertension. Affected persons all exhibited the leucine-for-serine substitution, had low plasma renin activities, and low aldosterone concentrations. Whereas the phenotype resembled Liddle syndrome, the investigators ruled out the presence of Liddle gene mutations. The authors speculate that the mineralocorticoid receptor gene mutation is an activating mutation in the receptor.

Interestingly, affected women exhibit a worsening of hypertension during pregnancy, suggesting that progesterone occupancy of the receptor results in activation rather than inhibition of aldosterone-like effects. Similarly, spironolactone makes the blood pressure elevation worse, rather than better. A complete report of this fascinating disease entity is eagerly awaited.

Liddle syndrome remains a hotbed of investigation. This condition is caused by mutations in the β or γ subunit of the amiloride-sensitive epithelial sodium channel (ENaC). Proving again that man can be an excellent model for hypertension in rodents, Pradervand and colleagues have constructed a mouse model that is almost as good as human patients [3•]. The investigators relied on a Cre/loxP-mediated recombination. Affected mice develop normally and have little hypertension despite low renin and low aldosterone concentrations. However, given a high salt diet, the mice develop high blood pressure, metabolic alkalosis, and hypokalemia. Animal investigations of ENaC function will be highly relevant to human hypertension. Additional examples include elucidation of the α ENaC subunit's role in channel expression, modulation by aldosterone, and salt balance [4].

Magnesium homeostasis is important to blood pressure regulation. Identification of paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption, may be highly important to the study of hypertensive mechanisms. A Mg^{2+} wasting syndrome was elucidated by Simon *et al.* [5••], who were able by positional cloning to identify *PCLN-1*, the paracellin-1 gene. The PCLN-1 protein is located in tight junctions of the thick ascending limb and is related to the claudin family of tight junction proteins. The findings provide insight into Mg^{2+} homeostasis, demonstrate a role of tight junction proteins in human disease, and identify an essential component of selective paracellular conductance.

Obesity-associated hypertension is the commonest form of secondary hypertension and is also influenced by genetic variance. The orphan nuclear receptor, peroxisome proliferator-activated receptor gamma ($PPAR\gamma$) is bound and activated by thiazolidinediones, medications useful in the treatment of type 2 diabetes mellitus. $PPAR\gamma$ activity is involved in lipid and carbohydrate metabolism, in addition to other fundamental intracellular processes. $PPAR\gamma$ mutations have been found in severe, monogenic obesity. Recently, dominant negative mutations in human $PPAR\gamma$ have been associated with severe insulin resistance, diabetes mellitus and hypertension [6••]. Knoblauch *et al.* [7] performed a combined linkage and association twin study and found that the $PPAR\gamma$ gene locus was linked to body mass index and

that a polymorphism in the gene was associated with body mass index and serum lipid concentrations in normal subjects. It is highly likely that the $PPAR\gamma$ gene and its binding partners will be interesting to students of hypertension.

Insight into *hypertension* may be obtained by understanding various forms of *hypotension*. A monogenic hypotensive condition has been mapped to chromosome 18q. DeStefano *et al.* [8•] identified a familial orthostatic hypotensive disorder. Positional cloning of the gene involved may shed important light on blood pressure regulation.

An additional monogenic hypotensive condition was reported [9] at last year's Association of Patient-Oriented Research annual meeting. Shannon *et al.* presented a family in whom they were able to identify a defect in a gene for a norepinephrine transporter.

Finally, Schwartz *et al.* [10] recently presented evidence that even mitochondrial DNA mutations may be responsible for orthostatic hypotension.

Human genetic linkage studies for new blood pressure loci and hypertension

In order to identify new genes responsible for blood pressure regulation and hypertension, their location must first be determined by linkage studies. Several important linkage studies have recently been conducted. Baima *et al.* [11] found evidence for linkage between essential hypertension and a locus on human chromosome 17q. This region is syntenic for rat chromosome 10, which contains a putative quantitative trait locus (QTL) for blood pressure. This investigation supports earlier French and British studies. Xu *et al.* [12] performed linkage studies in concordant and discordant Chinese sib-pairs. The group reported mapping of a blood pressure QTL to chromosome 15q in the Chinese population (maximum lod score = 3.77). In a second duplication set, they found weak evidence confirming their findings. However, in a genome-wide scan of extreme sib-pairs, the authors were not able to find regions achieving a 5% genome-wide significance level [13]. In this study, blood pressure was classified into age-adjusted deciles. In another study of Chinese hypertensive sib-pairs, Niu *et al.* [14] focused on a series of candidate gene loci, including angiotensinogen, renin, ENaC subunits, adducin and kallikrein. No evidence for linkage between the phenotype hypertension and these gene loci could be found.

Wong *et al.* [15••] relied on linkage to analyze the ENaC subunit gene loci. They studied 286 white families from Victoria, Australia. Each family comprised both parents

and at least two natural children. All participants were genotyped at chromosome 16p12 with microsatellite markers. The investigators found strong evidence for linkage between systolic blood pressure after parametric and nonparametric analysis. Nagy *et al.* [16•] relied on monozygotic and dizygotic twins to show how genetic variance affects blood pressure and heart size. They then recruited the parents of the dizygotic twins to conduct an identity by descent (IBD) linkage analysis. They also found that chromosome 16p12 is a QTL for blood pressure in normal man. In addition, they found that blood pressure QTL exist at the IGF-1, the angiotensin (AT1) receptor, and the renin gene loci in man. The group has also recently identified the adducin gene locus as a QTL for blood pressure [17]. Takami *et al.* [18] relied on the same family-based study as Wong *et al.* [15••] to search for linkage between the endothelial nitric oxide synthase gene locus and blood pressure. However, they were not able to find evidence for linkage.

Finding QTLs for blood pressure by studying the loci of known candidate genes or identifying new QTLs is a long way from cloning new genes responsible for a complex genetic disease. This feat has rarely been accomplished in rats. Although I had earlier argued that no gene related to hypertension had ever been cloned on the basis of a linkage analysis in rats I may have been proved wrong in that assumption. Aitman *et al.* [19••] recently identified *Cd36* (also known as *Fat*) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in spontaneously hypertensive rats (SHRs). First, a QTL was identified. The authors then combined the use of cDNA microarrays, congenic mapping, and radiation hybrid mapping, to identify the defective gene. They found a mutation in *Cd36*, the gene that encodes fatty acid translocase. The authors concluded that *Cd36* deficiency underlies insulin resistance in SHRs. However, the human *Cd36* has already been described and no known relationship to blood pressure has been reported. Nevertheless, Pravenec *et al.* [20•] studied the issue further. They adopted a congenic approach to show that the region on chromosome 4 containing *Cd36*, when moved from a Brown Norway background to SHRs, ameliorated a number of cardiovascular risk factors, including high blood pressure. However, a deletion variant of *Cd36* was not critical to the initial selection of hypertension in SHRs. Human *Cd36* has been known for years and has not been implicated in hypertension to my knowledge.

Association studies

Most studies into the molecular genetics of hypertension have relied on association. The methodology is simple: practically anyone with a PCR machine, funding for reagents, and access to DNA samples from cases and

controls can rapidly generate genotypic data for an association study. In the last year, over 100 such studies on one or another aspect of hypertension or blood pressure have been reported. Unfortunately, the contribution of most association studies to our understanding of hypertension and its genetic determinants has been modest. Recently, criteria have been suggested for 'high quality' association studies. Reviewing these criteria is worthwhile because we can rest assured, the reviewers judging subsequent association studies will review them carefully [20••]. These criteria are:

- large sample size
- small *P*-values
- biological plausibility
- functional significance
- independent replication in several populations
- confirmation in family-based studies
- high odds ratios and/or high attributable risk.

The reader is invited to analyze the following association studies in terms of the above criteria.

Renin-angiotensin system genes

The angiotensin converting enzyme (ACE) insertion/deletion polymorphism has been perhaps the most common mutation associated with cardiovascular disease in the general population. However, associating this mutation to blood pressure has been difficult. Turner *et al.* [21] studied 988 female and 887 male subjects from Rochester, MN. They performed a detailed statistical analysis to deal with confounders. The results were by no means straightforward. The authors concluded that the influence of variations in the ACE gene on inter-individual variation in blood pressure is dependent on contexts that are indexed by gender, age and measurements of body size.

In the mean time, the ACE gene has been sequenced in 11 individuals so that its variants are known. The insertion/deletion polymorphism is an *Alu* repeat in intron 16. Rieder *et al.* [22••] recently showed that 78 varying sites were present in the ACE gene, that resolved into 13 distinct haplotypes. Of the variant sites, 17 were in linkage disequilibrium with the *Alu* repeat in intron 16. This incredible degree of diversity in a single gene gives us some idea of the challenge ahead.

The angiotensinogen gene has been an attractive candidate gene for hypertension. Fardella *et al.* [23] studied the A₆G variant, which is in linkage disequilibrium with the M235T polymorphism. They studied 191 hypertensive patients. The A variant was detected in 70% of alleles, while the G variant was present in 30% of alleles. Plasma aldosterone was higher in patients homozygous for AA than in those homozygous for GG. Urinary aldosterone excretion corresponded to these

findings. The authors suggested that the presence of the A variant could determine the appearance of hypertension through higher transcription of angiotensinogen and concomitant aldosterone production. However, no angiotensinogen results were reported and the authors were silent on any blood pressure-related effects. Schorr *et al.* [24] studied the angiotensinogen M235T variant and salt sensitivity in young normotensive subjects. They found that the AGT 235T allele is significantly associated with a positive family history of hypertension, but is not an important determinant of salt sensitivity. Kato *et al.* [25•] examined the M235T issue in Japanese subjects. They performed their own association study of 1232 individuals and also performed a meta-analysis on six previously reported studies. The results suggested that the M235T polymorphism is not of great importance in the Japanese. Also, Katsuya *et al.* [26] investigated the effects of aging in a small study of young and old Japanese with respect to ACE insertion/deletion, AGT M235T, and the angiotensin II receptor AT1 A1166C variations. No significant associations were found, nor was there evidence for age-associated effects.

Finally, the 'discoverers' of M235T discuss seven lessons they have learned from studying this polymorphism and also polymorphisms in the ENaC [27•]. The lessons are worth summarizing:

1. many studies on these issues are too weak;
2. populations vary;
3. phenotyping is not easy and often not accurate;
4. the causal relationships between molecular variants and hypertension are difficult to establish;
5. the contributions of rodents to human disease should be re-evaluated;
6. most molecular variants will have minor effects;
7. no dietary or treatment recommendations can be made today on the basis of genotype information.

However, the angiotensinogen gene is also complicated; perhaps newly elucidated mutations will advance this field [28].

ENaC-related genes, adducin, and 11 β -hydroxysteroid dehydrogenase

Ambrosius *et al.* [29] investigated genetic variants in ENaC in relation to aldosterone and potassium excretion and risk for hypertension on 249 young white individuals and 181 young black individuals. Of the five variants they studied, all but one were more common in black individuals than white individuals. G442V in the beta-subunit was present in 29 (16%) black individuals and only one white. This variant was associated with greater sodium retention and lower aldosterone values. However, the variant could not be associated with hypertension in an older cohort. Expression of variants in *Xenopus* oocytes did not result in changes in basal Na⁺ current.

Persu *et al.* [30] used single-strand conformation polymorphism to screen samples from 245 normal and 453 hypertensive patients. The search was expanded to a subset of 65 patients with low renin hypertension. Four neutral polymorphisms were detected. However, the variants were found in equal numbers in normotensive and hypertensive subjects. Regulators of ENaC have also received attention. The ENaC subunits contain PY motifs that are deleted in Liddle syndrome. Recent studies demonstrate that Nedd4 is a negative regulator of the ENaC [31]. However, any role for Nedd4 in hypertension remains to be defined.

Considerable work has been done on the cytoskeletal protein, adducin. Ferrandi *et al.* [32] recently advanced knowledge in this area. They found that both rat and human adducins stimulate Na⁺-K⁺-ATPase activity. In rats, adducins increase the ATP affinity for Na⁺-K⁺-ATPase. The mechanism of action involves a selective acceleration of conformational change in various pump proteins. Mutant human and rat adducins have higher affinities than wild-type adducins. The observations may be relevant to salt sensitivity. Nevertheless, the results of association studies involving adducin have been conflicting. Glorioso *et al.* [33•] recently tried to explain the discrepancies. They studied 490 hypertensives and 176 normotensives from Sassari, Italy and 468 hypertensives and 181 normotensives from Milan, Italy. The persons were genotyped for the Gly460Trp polymorphism. A positive association was found in the Milan cohort, but not in the Sassari cohort. However, plasma renin activity was lower and the blood pressure response to diuretics greater in persons carrying at least one 460Trp allele, compared to those not having this allele at both study sites.

The 11 β -hydroxysteroid dehydrogenase gene is mutated in apparent mineralocorticoid excess [1]. The enzyme metabolizes cortisol to cortisone, so that the hormone cannot occupy the mineralocorticoid receptor. The implications in terms of sodium-related hypertensive mechanisms are obvious. However, 11 β -hydroxysteroid dehydrogenase may influence blood pressure by other mechanisms as well. Hatakeyama *et al.* [34] provided evidence that 11 β -hydroxysteroid dehydrogenase may also be important to vascular wall function. Moreover, Lovati *et al.* [35••] provided very interesting information implicating 11 β -hydroxysteroid dehydrogenase in salt sensitivity of blood pressure. They studied 37 salt-sensitive and 112 salt-resistant normotensive persons. They showed that salt-sensitive persons excreted a ratio of cortisol to cortisone in their urine consistent with a reduced 11 β -hydroxysteroid dehydrogenase activity. They then used a polymorphic microsatellite marker in the gene and showed an association between a marker variant and salt sensitivity

of blood pressure. This genetic study is the first to show convincing evidence concerning the mechanism of salt sensitivity and suggests that 11 β -hydroxysteroid dehydrogenase variants could serve as a genetic marker for the condition.

On the other hand, claims for the glucocorticoid receptor gene and hypertension could not be confirmed. Lin *et al.* [36] examined DNA from the so-called '4-corners' study. They studied a polymorphism in the gene to show association to high blood pressure and microsatellites to establish linkage of the gene locus to blood pressure. For the groups as a whole, no evidence for association or linkage was found. However, in gender subgroups, weak evidence for association to blood pressure was identified.

G proteins

The putative role of G protein beta3 subunit 825T allele and hypertension has recently been reviewed elsewhere [37]. Baumgart *et al.* [38•] reported that carriers of 825T alleles more commonly exhibited ischemic events in response to an alpha(2)-adrenergic receptor agonist treatment during coronary angiography than those who did not carry this allele. Siffert *et al.* [39] recently identified an association between the 825T allele and obesity in a large, worldwide study. Jacobi *et al.* [40] studied a small number of subjects in considerable detail and found that the 825T allele was associated with impaired left ventricular filling in hypertensive subjects. Dong *et al.* [41•] studied 428 men and women of African origin, 40% of whom were hypertensive. They found a three-fold higher risk of hypertension among the carriers of the T variant both as heterozygotes and homozygotes. The estimate of effect and the blood pressure values in the groups carrying the T variant suggested a dominant model for the T allele. Their study showed a high frequency of the 825T allele in black people, and provides evidence that the T allele may be a susceptibility factor for the development of hypertension in black people. Given the high frequency of the T allele, even a two-fold increased risk of hypertension among the carriers of the T allele might account for 44% of the cases of hypertension in black people.

Jia *et al.* [42] examined whether or not the *GNAS1* locus, encoding the Gs α subunit is implicated in hypertension. They found a silent polymorphism (ATT->ATC, Ile¹³¹) in exon 5 of the *Gs α* gene. They then studied this polymorphism in 231 control subjects and 268 hypertensive persons. In untreated hypertensive persons, the polymorphism was related to systolic blood pressure, but not in normotensive individuals. In a multiple regression analysis performed in a beta blocker-treated subgroup, the *Gs α* genotype was the sole independent predictor of blood pressure response to beta blockade.

Endothelium-related factors

Takami *et al.* [17] could find no evidence that the nitric oxide synthase (NOS) gene locus may be linked to hypertension. Kato *et al.* [43] studied 1165 persons and examined the relevance of the Glu298 variant in the endothelial NOS gene. They found no support for any associations between the variant, hypertension, or blood pressure. Glenn *et al.* [44] studied inducible NOS. They tested NOS2A markers for association and linkage with hypertension in affected Australian Anglo-Caucasians. No evidence supporting a relationship with blood pressure was found. Nevertheless, Herlitz *et al.* [45] found that urine flow in response to L-arginine infusions in normal subjects was significantly less pronounced in persons with a positive family history of hypertension, compared to those with a negative family history.

The Lys198ASn polymorphism in the endothelin-1 gene has been the subject of an association study. Tiret *et al.* [46] found that the polymorphism was associated with blood pressure levels in overweight people. Prostacyclin is a strong, endothelium-derived vasodilator and Iwai *et al.* [47••] detected a repeat polymorphism in the human prostacyclin synthase gene. This variant was tested for functional significance in human endothelial cells and was found to influence promoter activity. The authors then performed a very convincing association study in 4971 Japanese participants. Systolic blood pressure, pulse pressure, and the odds ratio of hypertension were all associated with the SS genotype of this polymorphism. The function of the glucagon receptor could conceivably influence vascular behavior on the basis of cAMP responses. A missense mutation (Gly40Ser) in exon 2 of the gene has been shown to exhibit reduced cAMP responses. Brand *et al.* [48] performed an association study of 741 French hypertensive persons and compared them to 412 normotensive controls. The polymorphism may represent a risk for hypertension in men, but apparently not in women.

Sympathetic tone: alpha and beta adrenergic receptors

Leptin has been implicated in sympathetic tone. Paolisso *et al.* [49] recently demonstrated that plasma leptin levels are associated with myocardial wall thickness in hypertensive insulin-resistant men. Ozata *et al.* [50] observed that human leptin deficiency caused by a missense mutation features decreased sympathetic tone. Thus, it is quite possible that variations of the leptin gene may be associated with differences in sympathetic tone and blood pressure. The issue has not yet been investigated in any detail.

Human alpha1B-adrenergic receptor polymorphisms have received attention. Büscher *et al.* [51] studied variability in phenylephrine response and essential hypertension. They searched for an effect of alpha1B-

adrenergic receptor polymorphisms, but were not able to find polymorphisms in the coding region of the gene which accounted for variability in phenylephrine responses. Experimental evidence suggests that salt loading induces hypertension via a neurogenic mechanism mediated by the alpha2-adrenergic receptors. Makaritsis *et al.* [52] studied genetically engineered mice and found that a full complement of alpha2B-adrenergic receptor genes was necessary to raise blood pressure in response to dietary salt loading, whereas complete absence of the alpha2C-adrenergic receptor subtype did not preclude salt-induced blood pressure elevations.

Baldwin *et al.* [53] investigated the alpha2B-adrenergic receptor in sib-pairs with hypertension. They found two major genetic forms of the receptor, differing by the presence of either 9 or 12 glutamic acid residues in the acidic domain of the third cytoplasmic loop. However, investigation of the pattern of this variation in hypertensive sib-pairs suggested that the alpha2B receptor locus did not contribute substantially to genetic susceptibility for essential hypertension.

Presynaptic alpha(2)-adrenergic receptors are distributed throughout the central nervous system and are highly concentrated in the brain stem, where they contribute to neural baroreflex control of blood pressure. Makaritsis *et al.* [54] conducted experiments in additional strains of genetically engineered mice and observed that the alpha(2A)-adrenergic receptor subtype exerts a sympatho-inhibitory effect, and its loss leads to a hypertensive, hyperadrenergic state. This particular receptor gene has not yet been subjected to detailed genetic studies in human to my knowledge.

The beta2 adrenergic receptor and its variants have received considerable attention. Earlier studies have shown that the Arg16->Gly variant is associated with hypertension. Recently, Gratzke *et al.* showed that a beta2 adrenergic receptor variant affects resting blood pressure and agonist-induced vasodilation in young Caucasians. They found that the Gly variant was associated with higher blood pressures. Furthermore, homozygous Gly16 subjects showed a significantly decreased vasodilation during a salbutamol infusion compared with Arg16/Arg subjects. These results are consistent with data from an association study of African-Caribbean individuals reported earlier, but at variance with an association study from the Bergen Blood Pressure Study [1]. The beta2 adrenergic receptor gene is relatively small, but nevertheless harbors five, functionally relevant polymorphisms. We found 15 polymorphisms in all, when the entire gene was sequenced in over 100 pairs of monozygotic and 100 pairs of dizygotic twin subjects (unpublished results). Four of the five variants that cause amino-acid

exchanges were present in our subjects. We performed extensive haplotype analyses on these variants in a combined linkage and association twin study [55•]. The data would appear to support the conclusions of the Bergen Blood Pressure Study. More importantly, they underscore the complexity of molecular genetic analyses when multiple variants must be considered simultaneously.

Coping with the conundrum

In my earlier review, I pointed out that Sir George Pickering had shown over 50 years ago, that the genetics of hypertension are complex [1]. He and his colleagues estimated that 25–30 genes were likely to be responsible for blood pressure regulation. It is likely that each gene variant may contribute less than 1 mmHg to the final phenotype. No wonder then that association study results are difficult to duplicate! Sequencing of the entire ACE gene in 11 persons has shown us the variability of a single gene. That study yielded 13 different haplotypes and 17 variants that are in linkage disequilibrium with the intron-based insertion/deletion polymorphism. The beta2 adrenergic receptor gene is much simpler but none the less has at least 15 variants. Imagine now dealing with a database of 30 such genes simultaneously to interpret hypertension or blood pressure regulation!

Recently, an industrial consortium announced plans to identify 300 000 single nucleotide polymorphisms (about three per gene) across the entire genome. These polymorphisms will greatly expand our power to conduct molecular genetic studies. However, we must then face other problems. Studies involving single polymorphisms in single genes, as in most of the reports discussed here, may become irrelevant because they address blood pressure variance of <1 mmHg in the population. Furthermore, examining only a single polymorphism in a gene may miss more functionally relevant variants or may miss associations, because variants in a single gene may not necessarily be in linkage disequilibrium with each other. In my view, we must now look at genes in terms of functionally interrelated networks. For instance, we may study genes involved in sodium reabsorption in the cortical collecting duct as a group. ENaC subunits, Nedd4, mineralocorticoid receptor, 11 β -hydroxysteroid dehydrogenase, adducin, components of the sodium pump readily come to mind. Alternatively, we may examine genes of the renin–angiotensin system as a group. Or we may study genes involved in adrenergic tone. The term ‘metabolic control analysis’ has been suggested for such endeavors [56••]. We have recently developed such an analysis for lipid metabolism relying on a series of differential equations and a neural network strategy (Knoblauch *et al.* unpublished results).

The problem we face has several components. First, we require prospective genetic models, sib-pairs and parents in the form of nuclear families, twin studies, trios for transmission disequilibrium test strategies, or large association studies. The persons must be carefully phenotyped for blood pressure and intermediary phenotypes. Second, we require a genotyping capacity that is log-fold greater than those currently available to clinicians. Compare 400 microsatellite markers to the prospect of dealing with 300 000 single nucleotide polymorphisms! On the horizon are multilocus genotyping assays, relying on novel technologies [57]. Third, we require a bioinformatics expertise that is currently unavailable. The expertise to perform complex haplotype analyses of single genes will have to be expanded to include many genes simultaneously. New models and strategies must be developed. Finally, we require the funding to perform such studies. Is the investment worth it? As a participant and an observer, I am hedging my bets.

Conclusion

The elucidation of monogenic syndromes has shed new light on the functions of the mineralocorticoid receptor influencing volume homeostasis, other nuclear receptors such as PPAR γ influencing obesity, diabetes, and hypertension, and paracellular transport processes influencing magnesium homeostasis. Both conceptual and technical progress has been made in approaching hypertension as a complex genetic condition. Future association studies will have to meet more stringent requirements in terms of robustness and functional significance of gene polymorphisms. The availability of a 300 000 genome-wide SNP map will dramatically improve both linkage and association approaches. Investigators will increasingly view genes in terms of networks involved in a particular function or series of functions. These approaches will require genotyping on a much larger scale and novel bioinformatics. However, only the best phenotyped patient and proband collectives will warrant the effort.

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