Corcoran Lecture

Cardiovascular Genomics and Oxidative Stress

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Abstract—The majority of modifiable cardiovascular risk factors are complex, polygenic, or at least oligogenic traits, with genetic and environmental determinants playing important roles in disease risk and its phenotypic expression. The Human Genome Project and subsequent mouse and rat genome data have provided powerful tools to commence the dissection of genetic determinants of hypertension and other cardiovascular risk factors. Despite several new methodologies such as genome-wide scans, genome-wide gene expression profiling, and proteomic screens, it is fair to say that the progress of genetic studies designed as nonhypothesis driven has been relatively slow. On the other hand, several interesting candidate pathways have been identified, where investigators allowed for hypothesis-driven functional studies. One example of such pathway is vascular oxidative stress with its extensive network of genes and proteins, many with proven contributions to cardiovascular disease. Therefore, in parallel to genome-wide or proteome-wide studies, it will be constructive to pursue “pathwayomics” defined here as functional studies of a candidate pathway for disease pathogenesis. (Hypertension. 2005;45[part 2]:636-642.)

Key Words: genetics ■ oxidative stress ■ endothelium

Human essential or primary hypertension is a substantial public health problem with >25% of the adult population being affected in industrial societies. Moreover, cardiovascular disease epidemic has now moved to the developing countries, with the projected increase in the proportion of all deaths attributable to cardiovascular causes from ~25% in 1990 to >40% in 2020.1 The cardiovascular continuum spans from risk factors such as hypertension, insulin resistance and type 2 diabetes, obesity, and hyperlipidemias through traits such as metabolic syndrome and atherosclerosis to the disease phenotypes including myocardial infarction, heart failure, stroke, peripheral vascular disease, and renal failure (Figure 1). It should be noted that all modifiable cardiovascular risk factors listed above are best described as complex, polygenic, or at least oligogenic traits with significant environmental influences, and thus the complexity facing us when investigating disease phenotypes attributable to target organ damage should not be underestimated.

We predict that the postgenome era, with its ability to study functions and interactions of all the genes in the genome, including their interactions with environmental factors,2 will bring improved understanding of cardiovascular complex traits. Furthermore, we predict that the classic genomic paradigms, including the central dogma of gene → protein → function as well as our increasing ability to study gene–gene and protein–protein interactions, will increasingly dominate mechanistic studies in hypertension and all other complex polygenic traits. This review will focus, at least initially, on genetics and genomics of essential hypertension, but similar strategies and considerations are applicable to other modifiable cardiovascular risk factors.

Study of Mendelian Disorders

There are at least 6 monogenic or Mendelian forms of hypertension, as summarized in Table 1. In these rare familial syndromes, there is a single gene mutation that explains the entire pathophysiological pathway, shows a clear Mendelian pattern of inheritance, and causes usually severe and early-onset hypertension.3–9 The analysis of monogenic forms of hypertension has yielded important pathogenic insights with a clear focus on mechanisms involved in the sodium and water reabsorption in the kidney.10 However, the syndromes described in Table 1 are very rare, and their contribution to the overall blood pressure variation in general population is small, if not negligible. Furthermore, studies that searched for more subtle polymorphisms in the same genes in patients with common essential hypertension have been either unsuccessful or difficult to reproduce.10 A possible exception here is the pseudohypoaldosteronism type II, also known as Gordon’s syndrome, a rare autosomal dominant hypertension associated with hyperkalaemia and easily treatable with thiazide diuretics. The genetic pathophysiology of this syndrome has been explained by Wilson et al,5 who have demonstrated that mutations in WNK1 and WNK4 genes are responsible for the final phenotype by increasing renal sodium and chloride reabsorption in the distal convoluted tubules and collecting ducts. This, in turn, contributes to the increase of intravascular volume and blood pressure. There is

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636
also some evidence from linkage and association studies that WNK4 may be involved in blood pressure regulation in subgroups of patients with essential hypertension.11,12

Rodent Models and Comparative Genome Analysis

For several years, rodent models of hypertension have been used successfully for the identification of quantitative trait loci (QTL). This strategy has been comprehensively reviewed by Rapp.13 The usual paradigm is to produce segregating populations derived from a hypertensive and normotensive strain and to seek linkage of blood pressure to genetic markers (usually microsatellite markers) using statistical programs such as MAPMAKER QTL or Map Manager.13 This approach has revealed candidate QTL regions on almost every rat chromosome as well as some important interactions between loci.13,14 However, most of these QTLs have been large (≈20 to 30 cM or more), and thus not suitable for classic positional cloning strategies. Several laboratories around the world proceeded to develop congenic strains to narrow down QTLs of interest.13,15,16 The progress toward cloning by positional identification of conserved synteny regions between rat, mouse, and human genomes has made it possible to use bioinformatic tools to transfer directly genomic discoveries between species.24 Moreover, regions common among various rat and mouse genetic crosses, also known as overlapping or reproducible QTLs, may represent target regions for genetic studies in man.15,24,25 The newly available bioinformatic tools make it possible to construct comparative maps for the identification of conserved synteny regions between rat, mouse, and human genomes.24,26 Such data could be combined with genome-wide microarray gene expression profiling and perhaps also proteomic and metabolomic analyses to provide functional data on the tens to hundreds of genes that can be mapped to a QTL or any congenic interval of interest. These genome-wide gene expression experiments have been done so far using 2 different strategies, which could be described as either “fishing expeditions” or hypothesis-driven research.27 The former experimental designs compare gene expression levels in experimental models of hypertension or animals with different degrees of target organ damage. These studies generate long lists of genes, which become somewhat uncertain candidate genes associated with the phenotype under study.27 Although novel genes and proteins contributing to cardiovascular disease might be discovered using the above strategy,28 it will remain difficult to prove causality in these experiments. To address the above problems, the

### Table 1. Mendelian Forms of Hypertension

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Mutation</th>
<th>Mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucocorticoid remediable alderosteronism (GRA)</td>
<td>Chimeric gene of aldosterone synthase and 11-β-hydroxylase</td>
<td>Aldosterone synthase gene expression regulated by adrenocorticotropic hormone instead of angiotensin II</td>
<td>3, 10</td>
</tr>
<tr>
<td>Liddle’s syndrome</td>
<td>Gain of function, mutations of the β and γ subunit of ENaC</td>
<td>Reduction in ENaC clearance and increased No. of channels and ENaC activity</td>
<td>4, 10</td>
</tr>
<tr>
<td>Gordon’s syndrome</td>
<td>Mutations in WNK1 and WNK4 genes</td>
<td>Increased reabsorption of chloride in the distal renal tubule</td>
<td>5</td>
</tr>
<tr>
<td>Pseudohypoaldosteronism type II (PHA II)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparent mineralocorticoid excess (AME)</td>
<td>Homozygous loss of function mutation of 11 β-hydroxysteroid dehydrogenase type 2</td>
<td>Reduced conversion of cortisol to cortisone</td>
<td>6</td>
</tr>
<tr>
<td>Hypertension accelerated by pregnancy</td>
<td>Missense mutation in the ligand binding domain of the mineralocorticoid receptor</td>
<td>Activation of receptor by progesterone</td>
<td>7</td>
</tr>
<tr>
<td>Hypertension with brachydactyly</td>
<td>Syndrome mapped to 12p11.2–12.2</td>
<td>Unknown, but a complex chromosome 12p rearrangement has been reported.</td>
<td>8, 9</td>
</tr>
</tbody>
</table>

Figure 1. Multiple interactions between genetic and environmental factors leading to the cardiovascular target organ damage; modified from reference 66.
second strategy aiming at a combination of classic genetic approaches with genome-wide expression profiling has been developed. This strategy applies gene expression profiling in genetically selected “designer strains” such as congenic or consomic rodent strains. Thus, instead of the expected hundreds or even thousands of genes that can be differentially expressed between 2 parental strains such as spontaneously hypertensive rats (SHR) and Brown-Norway or stroke-prone spontaneously hypertensive rats (SHRSP) and Wistar-Kyoto rats (WKY), these focused experiments will yield very few or even single genes that are not only differentially expressed but also map back to the congenic region. For example, identification of the \( Cd36 \) gene as a major contributor to insulin resistance and dyslipidemia in SHR followed this latter strategy, and this was further confirmed by a rescue transgenic experiment.

Recent data from our laboratory confirmed the feasibility of such an approach by comparing the SHRSP, its normotensive reference strain WKY, and a congenic strain, in which the QTL on chromosome 2 was introgressed from the WKY onto a hypertensive SHRSP genetic background. Total RNA was extracted from the kidney and hybridized to 26 379 genes and expressed sequence tags (ESTs) spotted on 3 chips (Affymetrix RGU34). Although there were 784 genes and ESTs differentially expressed between the 2 parental strains, there were only 45 probe sets with significant differential expression between SHRSP and the congenic strain. Of these, 3 probe sets mapped to the congenic region on chromosome 2 and all 3 were identified to correspond to the same gene: glutathione \( S \)-transferase \( \mu \) type 1 (\( Gstm1 \)). This gene lies on the important pathway of endogenous cellular defenses against oxidative stress, and thus its significantly lower expression in hypertensive compared with normotensive and congenic strains is likely to result in increased levels of reactive oxygen species (ROS) in the kidney and vascular tissue. Further studies in rat and man, including detailed functional analyses, are currently in progress, but it might be suggested that the increased generation of ROS combined with the lack of adequate defense mechanisms might be one of important pathophysiologic mechanisms involved in hypertension and its vascular complications. Moreover, oxidative stress or any other pathway identified in rodent models can be used in well-phenotyped human studies as a candidate pathway to define risk haplotypes. This might be particularly interesting because increased ROS have been implicated in all other cardiovascular risk factors, including diabetes, obesity, and hyperlipidemias, as summarized in a recent review. On a more fundamental physiological level, cellular effects of free radicals represent the most likely contender to explain the aging process across a wide range of species, and animal studies have demonstrated that the increased oxidative stress of hypertension could be seen as accelerated vascular aging. Thus, the cardiovascular continuum, as shown in Figure 2, could be fueled by the imbalance between ROS generation and disposal in all cardiovascular cells and tissues.

**Candidate Gene Evaluation**

This strategy has been used widely in genetics of hypertension and cardiovascular disease, with the most common study design being a case-control association study. In the context of genetic studies, the association is defined as the occurrence of a particular allele of a polymorphism in a group of patients more frequently than expected by chance. It is important to clearly distinguish association studies from linkage studies, in which genetic linkage is defined as coinheritance of genetic markers with the disease phenotype in families. The consistent coinheritance of the marker with the disease indicates that it is in close proximity (“linked”) to the disease gene.
Detailed analysis of all candidate genes that have been studied in human essential hypertension is beyond the scope of this review, but the “usual suspects” include genes within the renin-angiotensin system, \(\alpha\)-adrenergic and \(\beta\)-adrenergic receptors, growth factors, as well as genes encoding enzymes and peptides involved in endothelial function and vasoactivity. The relevant data have been summarized in recent reviews.\(^{43,44}\) It should be noted that a significant problem with many published association studies is that a positive association observed in one report is often not reproduced in subsequent studies.\(^{44}\) There has been a concerted effort to put forward criteria to achieve appropriate statistical power and reproducibility of candidate gene studies.\(^{45}\) In addition, the identification of millions of single nucleotide polymorphisms (SNPs; or variants at a single DNA bp) have allowed for the identification of groups of SNPs that travel together in families and populations. These groups are called haplotype blocks, each characterized by a relatively small number of SNPs: so called “tag-SNPs.”\(^{46}\) Despite these major advances and improved genetic markers, the issues related to statistical power remain a problem. Future studies should have sufficient sample size to detect small genetic effects and gene–gene and gene–environment interactions. One such study, the UK Biobank, is currently in advanced planning stages, and despite numerous criticisms,\(^{47}\) it seems very well poised to become one of the best DNA resources for candidate gene studies in cardiovascular and other complex traits. The second strategy to tackle candidate genes is to limit one’s interest to the positional candidates that are currently emerging from genome-wide scans (see below) or from comparative mapping strategies between rodent and human genomes, as described above.

**Genome-Wide Scans in Human Essential Hypertension**

The genome-wide scan is best defined as a search for QTL across the entire genome. In contrast to candidate gene studies described above, a genome scan is designed primarily to detect genes not implicated previously in pathways related to blood pressure variation. The entire genome is screened with densely distributed microsatellite markers to detect linkage of groups of markers with blood pressure or other traits of interest. A recent brief review summarized all genome scans for hypertension and blood pressure regulation published before and during 2003.\(^{48}\) The majority of studies used sibling pairs either concordant or discordant for hypertension. However, other study designs, such as nuclear families, large pedigrees, and studies of normotensive sibling pairs or families, have also been used. Table 2 provides a summary of genome scans published after submission of review by Samani\(^{48}\) and covering late 2003 and 2004 through October 1.\(^{49–62}\) Combined results of all these studies point to several suggestive and a few significant QTLs, with a substantial overlap between studies. The British Genetics of Hypertension (BRIGHT) study has been one of the largest, having genotyped 2010 severely hypertensive sibling pairs.\(^{56}\) This study identified a chromosomal region with a significant logarithm of odds (lod) score (chromosome 6q) and 3 suggestive lod scores (chromosomes 2q, 5q, and 9q). Computer simulations for a locus-counting analysis suggest that these linkages collectively attain genome-wide significance.\(^{56}\)

A possible interpretation of these findings is that human essential hypertension has an oligogenic rather than truly polygenic character, and several genes may be identifiable by further saturation of the QTLs with tag-SNPs for linkage disequilibrium mapping. Such strategies are currently in progress in several laboratories with emerging positive data for the chromosome 2 QTL in the Family Blood Pressure Programme.\(^{63}\) Moreover, recent meta-analysis\(^{64}\) showed evidence of significant or highly suggestive linkages on chromosomes 13p14.1-q12.3 and 2p12-q22.1 to hypertension and diastolic blood pressure. There are also important successes in searching for common polymorphisms contributing to stroke and myocardial infarction in the Icelandic population.\(^{65–67}\) It is likely that in the future, major research efforts will focus on positional and functional candidate genes discovered either by tightening the grid of markers and saturating with tag-SNPs within the QTLs or by translating robust rodent candidates to human family and population studies.

**Future Plans and Considerations**

The availability of sequence data of human, rat, and mouse together with major recent developments in 3 “omics” (that is, genomics, proteomics, and metabolomics) will lead to an accelerated progress toward detailed dissection of complex traits such as hypertension. A number of important considerations are necessary to support these developments. First, it will be essential to ensure that all human and rodent studies use standardized high-fidelity phenotyping methods for all cardiovascular phenotypes of interest. For example, more and more rodent studies will use radiotelemetry for blood pressure monitoring, whereas human investigations will rely on ambulatory blood pressure monitoring results. Other methods will include cardiac MRI for humans and rodents alike as well as reproducible blood and urine markers of oxidative stress and metabolic syndrome. Second, there is a need to develop a new set of statistical criteria as summarized by Thomas and Clayton,\(^{68}\) “. . . as we move into the era of genetic dissection of complex traits, we must abandon statistical criteria based on the incorrect assumption that a single genetic mutation is the necessary and sufficient cause of disease. Instead, we must think of a web of causation involving multiple and complex pathways.” Third, we have to develop rapid and accurate methods to test subtle polymorphisms in regulatory regions of rodent and human genes, which are likely to be discovered as important contributors to complex cardiovascular traits. Finally, we should not forget lessons learnt from monogenic traits.\(^{10}\) The accepted wisdom that the common alleles contribute to common traits has been challenged recently in a study by Cohen et al\(^{69}\) demonstrating that rare DNA sequence variants with major phenotypic effects contribute significantly to low-plasma HDL cholesterol levels in the general population.

**Perspective**

Provided we allow an appropriate time frame to fulfil all the above expectations, cardiovascular genomics is more than
### TABLE 2. Genome Scans for Blood Pressure and Hypertension: An Update

<table>
<thead>
<tr>
<th>Study</th>
<th>Name/Acronym</th>
<th>Setting</th>
<th>Phenotype</th>
<th>Participants</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiel et al 49</td>
<td>GenNet</td>
<td>USA</td>
<td>SBP; DBP</td>
<td>514 pairs (sib or half-sib) in 211 black families; 394 pairs (sib or half-sib) in 160 white families</td>
<td>1q (168–170 cM) (DBP, whites); 11q (76 cM) (SBP, whites); 3q (119 cM) (DBP, whites)</td>
</tr>
<tr>
<td>Rao et al 50</td>
<td>HyperGEN</td>
<td>United States</td>
<td>Hypertension</td>
<td>3403 individuals (1818 ASPs) in 489 white and 599 black families</td>
<td>2p (63 cM; blacks)</td>
</tr>
<tr>
<td>Kardia et al 51</td>
<td>GENOA</td>
<td>United States</td>
<td>Hypertension</td>
<td>989 ASPs in 229 black and 251 white families with hypertension diagnosed before age 60 y</td>
<td>1q (143 cM; whites)</td>
</tr>
<tr>
<td>Ranade et al 52</td>
<td>SAPPHIRe</td>
<td>United States</td>
<td>Concordance or discordance for hypertension or low BP</td>
<td>1123 Chinese sibpairs and 302 Japanese sibpairs (661 concordant for hypertension, 184 concordant for low BP and 580 discordant)</td>
<td>9q (163 cM; low BP); 10p (30 cM) (concordant and discordant sibpairs); 14q (92 cM) (hypertension)</td>
</tr>
<tr>
<td>Gong et al 53</td>
<td>China</td>
<td>Hypertension</td>
<td>94 individuals from one extended family (48 hypertensives) and 174 individuals from 32 nuclear families with hypertensive 12p (40 cM) offspring</td>
<td>12p (40 cM)</td>
<td></td>
</tr>
<tr>
<td>Von Wowern et al 54</td>
<td></td>
<td>Sweden</td>
<td>Hypertension</td>
<td>243 white individuals from 91 sibships with hypertension (&gt;160/90 mm Hg) diagnosed before age 50 y</td>
<td>2q (118 cM); 14q (41 cM)</td>
</tr>
<tr>
<td>Camp et al 55</td>
<td>Utah Center for Hypertension Detection &amp; Follow-up Program</td>
<td>United States</td>
<td>Pulse pressure</td>
<td>1454 white individuals from 26 extended families</td>
<td>8p (15.7 cM); 12q (20 cM)</td>
</tr>
<tr>
<td>Caulfield et al 56</td>
<td>BRIGHT</td>
<td>United Kingdom</td>
<td>Hypertension</td>
<td>2010 white ASPs (&gt;150/100 mm Hg) diagnosed before age 50 y</td>
<td>2q (141 cM); 5q (76 cM); 6q (190 cM); 9q (141 cM)</td>
</tr>
<tr>
<td>Morrison et al 57</td>
<td>Family Blood Pressure Program</td>
<td>United States</td>
<td>Hypertension</td>
<td>Unreported No. of non-obese siblings from 275 black families</td>
<td>2q (230 cM)</td>
</tr>
<tr>
<td>James et al 58</td>
<td>Framingham Heart Study</td>
<td>United States</td>
<td>Longitudinal SBP</td>
<td>1308 white siblings from Framingham Cohort 2</td>
<td>1q (192 - 202 cM)</td>
</tr>
<tr>
<td>Jacobs et al 59</td>
<td>Framingham Heart Study</td>
<td>United States</td>
<td>Longitudinal SBP and temporal SBP trend</td>
<td>2803 white sibpairs from 330 families</td>
<td>12q (78 cM) (mean SBP); 15q (129 cM) (mean SBP); 17q (109 cM) (mean SBP); 20q (74 cM) (SBP curvature)</td>
</tr>
<tr>
<td>Harrap et al 60</td>
<td>Victorian Family Heart Study</td>
<td>Australia</td>
<td>Postural changes in SBP; postural changes in DBP</td>
<td>274 white adult sibpairs</td>
<td>12q (69 cM)</td>
</tr>
<tr>
<td>DeStefano et al 61</td>
<td>Framingham Heart Study</td>
<td>United States</td>
<td>Pulse pressure</td>
<td>1702 white individuals from 330 families</td>
<td>5q (53 cM); 7q (71 cM); 10q (81 cM); 15q (122 cM)</td>
</tr>
<tr>
<td>Wilk et al 62</td>
<td>HyperGEN</td>
<td>United States</td>
<td>Hypertension; age at onset of hypertension</td>
<td>1095 white individuals from 422 sibships; 1161 individuals from 505 black sibships. Diagnosis of hypertension before age 45 y</td>
<td>1q (123 cM) (age at onset, whites); 18q (69 cM) (hypertension, whites); 4q (120 cM) (age at onset, blacks); 15q (60 cM) (age at onset, blacks); 4q (153 cM) (hypertension, blacks)</td>
</tr>
</tbody>
</table>

This table summarizes studies from 2003 to October 1, 2004, and provides an update of a previous summary by Samani. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; ASP, affected sibpair; y, years. Results in bold indicate genome-wide significance.
likely to produce clinical and public health dividend. This will include mechanistic classification of the common cardiovascular phenotypes, diagnostic markers leading to prophylactic medicine, the identification of targets and pathways for novel therapeutic interventions and tailoring of particular treatments to patients who are most likely to benefit on the basis of individual cardiovascular risk haplotypes.

Acknowledgments

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References


