Abstract—Human essential hypertension is a complex, multifactorial, quantitative trait under a polygenic control. Several strategies have been developed over the last decade to dissect genetic determinants of hypertension. Of these, the most successful have been studies that identified rare mendelian syndromes in which a single gene mutation causes high blood pressure. The attempts to identify multiple genes, each with a small contribution to the common polygenic form of hypertension, have been less successful. Several laboratories focused their attention on rat models of genetic hypertension, which can be considered as a reductionist paradigm for human disease. Using numerous crosses between hypertensive and normotensive strains, investigators identified several quantitative trait loci (QTL) for blood pressure subphenotypes and for cardiovascular complications such as left ventricular hypertrophy, kidney failure, stroke, and insulin resistance. Furthermore, congenic strains have been produced to confirm the existence of some of these QTL and to narrow down the chromosomal regions of interest. A number of interesting strategies have been developed, including a “speed” congenic strategy perfected by our group in Glasgow. However, the limit of congenic strategy is estimated at 1 cM, which corresponds to $2 \times 10^6$ base pairs of DNA and $\approx 50$ candidate genes. It is envisaged that gene expression profiling with cDNA microarrays might allow a quick progression toward the gene identification within cardiovascular QTL. In parallel experimental effort, several laboratories have been developing gene transfer/therapy strategies with adenoviral or adeno-associated viral vectors used, for example, to overexpress protective vascular genes such as vascular endothelial growth factor or endothelial nitric oxide synthase. It is anticipated that further developments in positional cloning of susceptibility and severity genes in hypertension and its complications will lead to a direct transfer of these discoveries to essential hypertension in humans and will ultimately produce novel targets for local and systemic gene therapy in cardiovascular disease. (Hypertension. 2000;35[part 2]:164-172.)

Key Words: genes ■ rats ■ stroke ■ endothelium ■ hypertension, essential

Human essential hypertension is a typical example of a complex, multifactorial, and polygenic trait. It is most likely that there are several causal genes, which together contribute to between 30% and 50% of the variation in blood pressure among individuals. These genetic determinants interact with environmental factors such as, for example, dietary salt to produce the final disease phenotype. Despite very significant recent progress in genomic and statistical tools, the genetic dissection of human essential hypertension still provides a major challenge. Three main lines of investigation have been developed so far. These include linkage analysis in families segregating for rare, mendelian forms of hypertension, candidate gene approaches, and genome-wide scanning strategies. Of the above approaches, the search for single-gene, mendelian forms of hypertension has been the most successful, resulting in several elegant publications illustrating how a single gene mutation leads to the high blood pressure phenotype. Major mutations have been identified with the use of this strategy in the following genes: 11β-hydroxylase in glucocorticoid remediable aldosteronism, the β and γ subunits of the epithelial sodium channel in Liddle’s syndrome, and the 11β-hydroxysteroid dehydrogenase in the syndrome of apparent mineralocorticoid excess. Two further mendelian forms of hypertension, Gordon’s syndrome and hypertension plus brachydactyly, have been mapped to defined chromosomal regions but their respective causal genes still await identification. It should be noted that these rare syndromes with mendelian inheritance account for a very small fraction of the pathological human blood pressure variation. However, the most popular candidate gene strategy, which investigates the genes within physiological pathways known to affect blood pressure variation, is a result of lessons learned while studying these rare syndromes.

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From the BHF Blood Pressure Group, Department of Medicine and Therapeutics, University of Glasgow, Scotland.

Correspondence to Prof Anna F. Dominiczak, BHF Blood Pressure Group, Department of Medicine and Therapeutics, Western Infirmary, Glasgow, Scotland. E-mail: ad7e@clinmed.gla.ac.uk

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The great majority of the candidate genes studied have been chosen from those encoding enzymes and peptides of the renin-angiotensin system or other proteins related to water and sodium handling. These candidate gene studies have been summarized in excellent recent reviews. Rather than repeat all the evidence for and against individual candidate genes, it is worth noting that after 8 years of intensive study, the angiotensinogen (AGT) gene emerges as the strongest contender, although its contribution to essential hypertension is probably small.

The genome-wide scanning strategy has been a subject of extensive methodological and statistical discussions for a number of years, but only recently early results on either concordant or discordant for blood pressure sib-pairs have been reported. Krushkal et al performed a genome-wide linkage analysis of systolic blood pressure in humans by using a highly discordant, full-sibling design. They identified linkage analysis of systolic blood pressure in humans by concordant or discordant sibs identified 5 chromosomal regions, 16,17 but none of the results have been fully reproducible.16,17

In the late eighties and throughout the nineties, several laboratories have made imaginative use of the existing inbred rodent models of genetic hypertension. These studies have resulted in the identification of several quantitative trait loci (QTL) responsible for blood pressure regulation, as summarized in recent comprehensive reviews. Careful analysis of these data reveals several points of special interest for all researchers of hypertension. First, 1 or more QTL have been identified on almost all rat chromosomes with the exception of chromosomes 6, 11, 12, and 15. Second, despite a great variability between the hypertensive strains and the genetic crosses used in QTL mapping, several QTL have been present reproducibly in many independent experiments, as shown schematically in Figure 1. These common or reproducible QTL have become important candidate loci for human essential hypertension. The best example is the QTL on rat chromosome 10, which is a classic reproducible QTL reported in the SHRSP x WKY, Dahl S x MNS, Dahl S x LEW, GH x BN, and SHR x WKY cross. Julien et al used this QTL to investigate the homologous region of conserved synteny on human chromosome 17. In their study of 518 sib-pairs concordant for essential hypertension, there was evidence of significant linkage to human chromosome 17q around 2 microsatellite markers. The same region has also been linked to pseudohypoaldosteronism type II or Gordon’s syndrome in a subset of families. More recently, Baima et al confirmed these data in another population of patients with essential hypertension and defined further the region of interest (Figure 2). These 2 studies provide an elegant proof of the concept that it is feasible to directly translate the QTL discovered in the rat to human essential hypertension and pave the way for similar studies for other cardiovascular QTL.

The third important observation concerns the interaction among alleles at different QTL or epistasis. For example, Rapp et al demonstrated a major interaction on blood pressure between the QTL on chromosomes 2 and 10 in an F2 cross and then confirmed this epistasis by a construction of a double congenic strain as described in detail below.

In the mid-nineties, several groups, including our own, embarked on studies designed to map QTL influencing other than high-blood-pressure cardiovascular phenotypes. These included studies on left ventricular hypertrophy, ischemic stroke, renal impairment and insulin resistance. It should be noted that this review will only discuss cardiovascular QTL, which have been shown to be at least partially blood pressure independent. These loci are summarized in Table 1. As documented by Rapp, blood pressure QTL are almost invariably associated with effects on heart weight; indeed, the presence of these effects has been used as a confirmation of blood pressure QTL data. However, several groups reported loci that are responsible for a proportion of left ventricular weight that were blood pressure independent (References 32 to 34 and Table 1). Unlike the striking reproducibility of blood pressure QTL, there have been no duplication of loci for left ventricular hypertrophy (LVH) in any of the rat crosses examined. Furthermore, Sekhri et al reported a QTL on rat chromosome 3 that accounted for 16.5% of the total variance of left ventricular weight in a cross between 2 normotensive strains (Fisher 344 and WKY), yet again confirming the existence of the genetic determinants of cardiac hypertrophy that are blood pressure independent. Regions on chromosomes 12 (close to Hsp 27) and X have been implicated to contribute to LVH in a blood pressure-independent manner in the WKY x SHR and LH x LN crosses, respectively (References 36 and 37 and Table 1). It seems, therefore, that there are several potential loci that might determine LVH in genetic hypertension but further evidence over and above statistically significant linkage is
As for blood pressure QTL, this will require production of congenic strains and substrains and a subsequent positional cloning of the causal genes. Compelling evidence has also been produced for at least 4 separate QTL that influence stroke in a blood pressure–independent fashion. Rubattu et al. identified 3 QTL for susceptibility to stroke in the SHR x SHRSP Hd cross. These QTL have been mapped to chromosomes 1, 4, and 5, with the QTL on chromosome 5 localized in close proximity to the gene encoding atrial natriuretic factor (Anf). In this study, the chromosome 5 locus appeared to protect from stroke in the SHRSP Hd as the SP/SP homozygotes had significantly greater latency to spontaneous stroke on a Japanese diet (high salt, low potassium, and low protein) than did SH/SH homozygotes. Our own data revealed a stroke severity locus in an F2 population obtained by crossing SHRSP Gla and WKY Gla. This QTL was localized to chromosome 5, had a highly significant linkage (LOD score of 16.6), accounted for 67% of the phenotypic variance, and was blood pressure independent. It should be noted that this QTL colocalizes with the blood pressure QTL observed in studies on Dahl S rats. It appears that there are 2 separate QTL for stroke on rat chromosome 5 and a blood pressure QTL that might be either related or unrelated to one of these stroke QTL. A current comprehensive review by Rapp provided a unifying speculation to explain the coexistence and colocalization of the blood pressure–independent stroke QTL and the blood pressure QTL on rat chromosome 5. It assumes that a vasoconstrictor response influenced by a locus on chromosome 5 at the renal or systemic level responds to high salt intake in the Dahl rat model. The same locus may affect cerebral collaterals and their response to brain ischemia on a normal salt diet and in a blood pressure–independent manner. Congenic experiments are currently in progress and are likely to either confirm or refute this hypothesis.

Studies on an animal model of hypertension and renal impairment in a back-cross between the fawn-hooded hypertensive rat and the normotensive ACI rat localized 2 QTL, Rf-1 and Rf-2, responsible for renal sclerosis and/or proteinuria. Both QTL mapped to rat chromosome 1, but only 1 of the loci, Rf-1, was blood pressure independent. These results demonstrated that susceptibility to hypertension and renal impairment are under at least partially independent genetic control. Further evidence for the existence of specific genetic determinants for the susceptibility to kidney damage comes from studies by Churchill et al., who developed a kidney-specific genome transfer. These studies demonstrated that the kidney of the normotensive Brown Norway (BN) rat is inherently more susceptible to hypertension-induced damage than is the kidney of the SHR.

Perhaps the most interesting recent results come from studies on insulin resistance phenotypes by Aitman et al. and Pravanec et al. Initial data showed 3 QTL responsible for the metabolic syndrome in the SHR x WKY cross. Two of these QTL were responsible for defective insulin action and were localized on chromosomes 4 and 12. The major genetic determinant of defective control of lipolysis in the

<table>
<thead>
<tr>
<th>Disease</th>
<th>Chromosome</th>
<th>Rat Strains (crosses)</th>
<th>Central Gene/Marker</th>
<th>LOD Score</th>
<th>Year, Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular hypertrophy</td>
<td>2</td>
<td>Donryu x SHR</td>
<td>D2Mgh15</td>
<td>4.3</td>
<td>1998, 34</td>
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<tr>
<td></td>
<td>12</td>
<td>WKY x SHR</td>
<td>Hsp27</td>
<td>n/a</td>
<td>1996, 36</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>WKY x SHRSP&lt;sub&gt;gla&lt;/sub&gt;</td>
<td>D14Mgh3</td>
<td>3.1</td>
<td>1996, 32</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>RI (BN.Lx x SHR)</td>
<td>Drd1a</td>
<td>n/a</td>
<td>1995, 33</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>LN x LH</td>
<td>n/a</td>
<td>n/a</td>
<td>1996, 37</td>
</tr>
<tr>
<td>Stroke</td>
<td>1</td>
<td>SHR x SHRSP&lt;sub&gt;hd&lt;/sub&gt;</td>
<td>D1Mit3</td>
<td>7.4</td>
<td>1996, 38</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>SHR x SHRSP&lt;sub&gt;hd&lt;/sub&gt;</td>
<td>D4Mgh16</td>
<td>3.0</td>
<td>1996, 38</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>SHR x SHRSP&lt;sub&gt;hd&lt;/sub&gt;</td>
<td>Anf</td>
<td>4.7</td>
<td>1996, 38</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>WKY x SHRSP&lt;sub&gt;gla&lt;/sub&gt;</td>
<td>D5Wox4</td>
<td>16.6</td>
<td>1997, 39</td>
</tr>
<tr>
<td>Renal impairment</td>
<td>1</td>
<td>ACI x FHH</td>
<td>D1Mit6</td>
<td>8.9</td>
<td>1996, 41</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>4</td>
<td>WKY x SHR</td>
<td>D4Arb13, Ae2</td>
<td>6.4</td>
<td>1997, 42</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>WKY x SHR</td>
<td>D12Mit18</td>
<td>6.3</td>
<td>1997, 42</td>
</tr>
</tbody>
</table>

The following are microsatellite markers: D1Mit3, D4Mgh16, D5Wox4, D2Mgh15, D14Mgh3, D1Mit6, D4Arb13, and D12Mit18. Other symbols represent markers within known genes: Anf, atrial natriuretic peptide; Drd1a, dopamine 1a receptor; Hsp27, heat shock protein. RI(BN.Lx x SHR) denotes recombinant inbred strains derived from the SHR and the normotensive BN rats. LN and LH are Lyon normotensive and hypertensive rat strains, ACI are (AxC9935 Irish) normotensive rats from Harlan Sprague Dawley, and FHH is the fawn-hooded hypertensive rat strain.
SHR mapped to the same region of chromosome 4, which suggests the possibility of a gene defect acting on the pathways that control both glucose and fatty acid metabolism.43 Further studies from the same laboratory, which combined several new strategies including cDNA microarrays combined with congenic mapping and radiation hybrid mapping, identified a defective SHR gene, Cd36, at the peak of linkage on rat chromosome 4.44 This gene also known as Fat, because it encodes fatty acid translocase, has multiple coding sequence variants in its cDNA, and its protein product is undetectable in SHR adipocyte plasma membrane. Together the data presented by Aitman et al43,44 produce compelling evidence for the role of Cd36 deficiency in the insulin resistance, defective fatty acid metabolism, and hypertriglyceridemia in the SHR. Pravanec et al45 demonstrated that transfer of a segment of chromosome 4 (including Cd36) from the BN rat into the SHR background induces reductions in blood pressure and ameliorates fructose-induced glucose intolerance, hyperinsulinemia, and hypertriglyceridemia. However, Cd36 genotyping performed in a closely related hypertensive strain, SHRSP did not reveal the deletion variant carried by the SHR.45 It follows that although Cd36 is a likely determinant of abnormalities in glucose and lipid metabolism in the SHR, its role in the pathogenesis of hypertension requires further study.

**Congenic Strategies for Fine Mapping of QTL**

As outlined above, several blood pressure and other cardiovascular QTL have been identified in experimental crosses. However, the identification of these relatively large chromosomal regions (on average 20 to 30 cM) is only the first step toward the ultimate goal of gene identification.20,46 The next

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Congenic Strain</th>
<th>Chromosomal Region Transferred, cM</th>
<th>Blood Pressure Change, mm Hg</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SHR.BN</td>
<td>22</td>
<td>−10</td>
<td>48</td>
</tr>
<tr>
<td>1</td>
<td>Dahl S.LEW</td>
<td>33</td>
<td>−26</td>
<td>26</td>
</tr>
<tr>
<td>1</td>
<td>WKY.SHR</td>
<td>26</td>
<td>+11</td>
<td>49</td>
</tr>
<tr>
<td>1</td>
<td>SHR.WKY</td>
<td>15</td>
<td>−11</td>
<td>49</td>
</tr>
<tr>
<td>1</td>
<td>WKY.SHR</td>
<td>27</td>
<td>+8</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Dahl S.WKY</td>
<td>38</td>
<td>−44</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>Dahl S.MNS</td>
<td>78</td>
<td>−29</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>Dahl S.Dahl R</td>
<td>17</td>
<td>−21</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td>SHR.BN</td>
<td>36</td>
<td>−15</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>Dahl S.LEW</td>
<td>32</td>
<td>−15</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>Dahl S.Dahl R</td>
<td>9.9–35.2</td>
<td>−40</td>
<td>53</td>
</tr>
<tr>
<td>8</td>
<td>SHR.BN (Lx)</td>
<td>31</td>
<td>−20</td>
<td>54</td>
</tr>
<tr>
<td>9</td>
<td>Dahl S.Dahl R</td>
<td>21</td>
<td>−19</td>
<td>55</td>
</tr>
<tr>
<td>10</td>
<td>Dahl S.MNS (10a)</td>
<td>31</td>
<td>−42</td>
<td>56</td>
</tr>
<tr>
<td>10</td>
<td>Dahl S.LEW</td>
<td>27</td>
<td>−42</td>
<td>26</td>
</tr>
<tr>
<td>13</td>
<td>Dahl R.Dahl S-renin</td>
<td>19</td>
<td>−8</td>
<td>57</td>
</tr>
<tr>
<td>13</td>
<td>Dahl S.Dahl R-renin</td>
<td>6</td>
<td>+27</td>
<td>58</td>
</tr>
<tr>
<td>13</td>
<td>Dahl S.Dahl R-renin</td>
<td>24</td>
<td>−24</td>
<td>59</td>
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<tr>
<td>19</td>
<td>SHR.BN</td>
<td>12</td>
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<td>60</td>
</tr>
<tr>
<td>2 and 10</td>
<td>SHR.WKY</td>
<td>Whole</td>
<td>−15</td>
<td>61</td>
</tr>
<tr>
<td>2 and 10</td>
<td>WKY.SHR</td>
<td>Whole</td>
<td>+35</td>
<td>61</td>
</tr>
</tbody>
</table>

The recipient strain is cited first and the donor strain second. Dahl R and Dahl S are Dahl salt-sensitive and Dahl salt-resistant rats; LEW is Lewis strain; and MNS is Milan normotensive strain. BN(Lx) is a donor strain originally derived by introgressing the mutant Lx gene of the polydactilous rat on to the normotensive BN background. Dahl S.MNS (10a) represents the congenic that produced the greatest blood pressure reduction from the several constructed (10a–d).
step is the production of congenic strains and substrains containing progressively smaller chromosomal regions, as illustrated in Figure 3.

A congenic strain is one in which the chromosomal region harboring the QTL of interest in one strain (the recipient) has been selectively replaced by the homologous region from another strain (the donor). If the blood pressure or any other quantitative trait of the congenic strain is significantly different from the recipient strain, it can be concluded that this particular chromosomal region does indeed possess a QTL that contributes to a phenotypic difference between the donor and recipient strains. Congenic strains have traditionally been produced by serially back-crossing the donor strain with the recipient strain, accompanied at every generation by selection for progeny heterozygous for the chosen chromosomal region. Between 8 to 12 back-crosses are necessary to ensure that ~99% of the donor’s genetic background has been replaced by that of the recipient. On completion of these back-crosses, brother-sister mating makes the desired chromosomal region homozygous for the donor’s alleles.

This relatively simple breeding strategy has resulted in the development of several rat congenic lines over the last 3 years, confirming the existence of QTL involved in blood pressure regulation on rat chromosomes 1, 2, 3, 4, 5, 7, 8, 9, 10, 13, and 19,62,63,48–60 and a consomic strain (that is, a strain in which the whole chromosome has been replaced) for the Y chromosome,61 as summarized in Table 2. However, these have taken on average 3 to 4 years to produce, a length of time that has significantly delayed the progress toward the identification of the genes involved.

Theoretical calculations by Lander and Schork62 suggested that congenic strain production may be “speeded up” by repeated screening of polymorphic marker loci distributed throughout the entire genetic background, thereby allowing the specific selection of a male from each back-cross with the least donor alleles remaining in the genetic background. Breeding with the “best” males would allow the rate of background donor elimination to be dramatically accelerated, thereby reducing the number of generations necessary to construct a congenic strain. Computer simulations have indicated that a relatively modest selection effort (60 background microsatellite markers, 25-cM marker spacing, 16 males per generation) would typically reduce unlinked donor genome contamination to <1% by 4 back-cross generations.63,64 The development of 10 “speed” congenic mice strains carrying defined genomic intervals derived from nonobese diabetic or NZM2410 strains on the C57BL/6 genetic background provided results that closely parallel these predicted outcomes.65,66 Recently we used a speed congenic breeding protocol for 2 blood pressure QTL on rat chromosome 2.67 Four congenic strains were produced by introgressing various segments of chromosome 2 from WKY rats into the recipient SHRSP strain and vice versa. The number of back-cross generations required for each strain to achieve complete homozygosity at 83 background genetic markers varied between 3 and 4. Transfer of the region of rat chromosome 2 from WKY into a SHRSP genetic background lowered both baseline and salt-loaded systolic blood pressure (measured by radiotelemetry) by ~20 and 40 mm Hg in male congenic rats as compared with the SHRSP parental strain (F=53.4, P<0.005 and F=28.0, P<0.0005, respectively). These results demonstrated, for the first time, the applicability of a speed congenic strategy in the rat.67
Other novel application of the traditional congenic strategy has been developed by Rapp et al.\textsuperscript{31} To test the hypothesis that there is an interaction between QTL on rat chromosomes 2 and 10, they developed a double congenic strain with both the chromosome 2 and 10 low blood pressure alleles on the hypertensive Dahl salt-sensitive (S) strain background. This study confirmed a significant interaction between chromosomes 2 and 10 on blood pressure accounting for 24 mm Hg (Table 2). Similar epistatic interactions are very likely to exist in human essential hypertension, yet again some aspects of rat cardiovascular genetics might be directly relevant to human studies.

Future Strategies in Experimental Genetics of Hypertension

The most crucial future aim is to positionally clone causal genes within all cardiovascular QTL identified. To achieve this, it will be essential to refine and to narrow down the existing QTL to \( \approx 1 \) cM, a requisite size at which positional cloning becomes feasible.\textsuperscript{46} The advocated method here is fine genetic substitution mapping, which requires high-density polymorphic markers and the ability to perform high-fidelity phenotyping at each strain.\textsuperscript{20} Once the region of interest has been saturated with new markers and narrowed down to the order of \( \approx 1 \) cM, physical mapping of the region is undertaken with yeast artificial chromosomes, bacterial artificial chromosomes, and a P1 bacteriophage vectors, also described as P1-derived artificial chromosomes.\textsuperscript{68–70} These are used in parallel to generate contigs (clusters of overlapping clones representing the contiguous DNA from a genomic region), initially by screening for clones containing the closest flanking genetic markers. The limit of this approach is the distance between the 2 closest recombination events in the congenic substrain.\textsuperscript{20} Once this limit is reached, an exhaustive search of the entire region is required to find coding sequences within the candidate region.\textsuperscript{71} If the markers are close on the chromosome, then the flanking yeast artificial chromosomes clones will overlap, as judged by pulsed field electrophoresis restriction patterns or other markers in common. The next step is to find expressed sequences and to assess them as candidates. One of the methods here is a cDNA selection, in which mRNA from the most likely tissues to be expressing the candidate gene is converted to cDNA and hybridized to genomic clones spanning the critical region. The efficiency and throughput of the latter methodology has recently been greatly improved by the development of cDNA microarrays with the ability to spot on a chip thousands of different DNA molecules.\textsuperscript{72} Aitman et al\textsuperscript{44} used a microarray with 10,000 cDNA clones derived from a normalized library of rat heart. They studied differential expression between the parental SHR and the SHR.4 strain. These 2 strains are identical apart from the 36-cM region at the peak of insulin resistance QTL on chromosome 4 (Reference 44 and Table 2). As expected, there were fewer differentially expressed clones in the SHR/SHR.4 comparison than in the SHR/BN comparison, thus allowing for a rapid identification of the causal gene for the insulin resistance phenotype, as described in detail above. It seems that well-defined congenic strains provide an ideal target for gene expression profiling with cDNA microarrays. This new strategy might allow us to bypass the more laborious and time-consuming traditional physical mapping methods.

Somatic Gene Transfer/Therapy in Cardiovascular Disease

Gene therapy is defined as the use of nucleic acids as therapeutically useful molecules. Its current place, promise, and problems have been recently reviewed.\textsuperscript{73,74} The gene therapy approaches have many potential applications, the most obvious being the correction of single gene mutations in monogenic diseases such as cystic fibrosis.\textsuperscript{75} However, other clinical applications are also possible and include specific gene transfer–based therapies for cancer and HIV infection, which, because of their life-threatening character, encourage a fast transition from experimental to clinical studies.\textsuperscript{74} In light of a steady progress toward the identification of causal genes in human and experimental hypertension and its vascular end-organ damage as outlined above, the time is ripe to consider appropriate gene transfer strategies for cardiovascular disease. The rationale for gene therapy aimed at lowering blood pressure in hypertension and the examples of experimental studies that have achieved this goal have been recently reviewed by Phillips.\textsuperscript{76} He argued that in analogy to powerful pharmacological tools such as \( \beta \)-blockers, angiotensin-converting enzyme inhibitors, and angiotensin type 1 receptor antagonists, gene therapy strategies aimed at increasing vasodilation or inhibiting vasoconstriction through genetic manipulation will precede full understanding of the genetic pathogenesis of essential hypertension. He argued further that gene transfer strategies will produce continuous biological control over high blood pressure in a manner superior to current drugs, with a single administration of a harmless recombinant virus producing hypertensive effects lasting several months and with minimal adverse effects.\textsuperscript{76} Before these ambitious aims could become feasible and clinically applicable, further experimental work is necessary to overcome such hurdles as poor efficiency and limited duration of the foreign gene expression as well as the inflammatory and immune responses, the latter being particularly prevalent with the use of the first generation adenoenoviral vectors.\textsuperscript{74} A careful analysis of the existing comparative data between the available vectors seems to suggest that the adenov-associated viral vectors are the best candidates for therapeutic long-term delivery of genes because of their safety, lack of immune and inflammatory response, the ability to infect all mammalian tissues, and their constant expression in some tissues for several months.\textsuperscript{76,77}

Although strategies for systemic gene therapy in hypertension require further mechanistic experimental studies, one can envisage an easier transfer to the clinic for local molecular therapeutic strategies aimed at reversing vascular complications of hypertension and/or atherosclerosis (References 78 and 79 and Figure 4). These local gene transfer strategies are based on the premise that the expression of modest amounts of a secreted gene product, in readily accessible cells for a short period of time, should be efficacious.\textsuperscript{74} This approach has been used extensively in normal or atherosclerotic vessels with the use of recombinant adenoviruses encoding isoforms
of nitric oxide synthase gene (eNOS, iNOS, and nNOS), as summarized in Reference 78, and isoforms of the superoxide dismutase gene (CuZn intracellular SOD, Mn mitochondrial SOD, and extracellular SOD).80,81 Our own data demonstrated that adenovirally mediated, in vivo overexpression of the eNOS gene corrected the relative nitric oxide deficiency and restored endothelial function in the SHRSP carotid artery as compared with the normotensive reference strain, WKY (Reference 79 and Figure 5). Moreover, recent study from Heistad’s laboratory82 demonstrated that the ex vivo gene transfer of adenovirus encoding eNOS gene to vascular rings of mice with targeted disruption (knockout) of this gene restored their relaxation to acetylcholine and a calcium ionophore, A-23187. These 2 studies show that vascular function can be significantly improved by gene transfer in vessels in which nitric oxide–dependent relaxation is severely impaired (SHRSP) or even completely absent (eNOS-deficient mice).

The second approach to local vascular gene transfer is to use gene therapy to promote revascularization of ischemic tissue in coronary artery disease and peripheral vascular disease (References 74, 83, and 84 and Figure 4). The factors used to promote angiogenesis, especially the vascular endothelial growth factor (VEGF) gene, are very potent and have a bystander effect on adjacent tissue, such that the gene need not be expressed in large amounts, nor in every cell. Moreover, the VEGF and similar factors need to be expressed for only a limited period; thus the existing vector technology is sufficient even for clinical trials.83,84

Conclusions

The last decade has brought a steady progress in our understanding of genetic determinants of human and experimental hypertension. With the Human Genome Project due to be completed before 2003 and with genomic resources for rat and mouse being developed at an increasing pace, we are likely to achieve positional cloning of the major susceptibility and severity genes for hypertension and its vascular complications. The identification of these genes will provide a mechanistic classification of the common hypertension phenotype, diagnostic markers for individuals and families who are at greatest risk of end-organ complications such as stroke, coronary artery disease, or chronic kidney disease, and, ultimately, will provide new pharmacogenomic and gene transfer strategies tailored to the underlying primary genetic abnormalities.

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References


36. Deleted in proof.


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Genes and Hypertension: From Gene Mapping in Experimental Models to Vascular Gene Transfer Strategies

Anna F. Dominiczak, Domingo C. Negrin, James S. Clark, M. Julia Brosnan, Martin W. McBride and M. Yvonne Alexander

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