Identification of Hypertension-Related Genes Through an Integrated Genomic-Transcriptomic Approach

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Abstract—In search for the genetic basis of hypertension, we applied an integrated genomic-transcriptomic approach to identify genes involved in the pathogenesis of hypertension in the Sabra rat model of salt-susceptibility. In the genomic arm of the project, we previously detected in male rats two salt-susceptibility QTLs on chromosome 1, SS1a (D1Mgh2-D1Mit11; span 43.1 cM) and SS1b (D1Mit11-D1Mit4; span 18 cM). In the transcriptomic arm, we studied differential gene expression in kidneys of SBH/y and SBN/y rats that had been fed regular diet or salt-loaded. We used the Affymetrix Rat Genome RAE230 GeneChip and probed >30 000 transcripts. The research algorithm called for an initial genome-wide screen for differentially expressed transcripts between the study groups. This step was followed by cluster analysis based on 2×2 ANOVA to identify transcripts that were of relevance specifically to salt-sensitivity and hypertension and to salt-resistance. The two arms of the project were integrated by identifying those differentially expressed transcripts that showed an allele-specific hypertensive effect on salt-loading and that mapped within the defined boundaries of the salt-susceptibility QTLs on chromosome 1. The differentially expressed transcripts were confirmed by RT-PCR. Of the 2933 genes annotated to rat chromosome 1, 1102 genes were identified within the boundaries of the two blood pressure QTLs. The microarray identified 2470 transcripts that were differentially expressed between the study groups. Cluster analysis identified genome-wide 192 genes that were relevant to salt-susceptibility and/or hypertension, 19 of which mapped to chromosome 1. Eight of these genes mapped within the boundaries of QTLs SS1a and SS1b. RT-PCR confirmed 7 genes, leaving TecTex1, Myadm, Lisch7, Axl-like, Fah, PRCl-like, and Serpinh1. None of these genes has been implicated in hypertension before. These genes become henceforth targets for our continuing search for the genetic basis of hypertension. (Circ Res. 2005;96:617-625.)

Key Words: linkage ■ DNA microarrays ■ transcripts ■ candidate genes ■ salt-susceptibility

The genetic basis of hypertension, a complex disease, remains elusive. Genetic mapping of quantitative trait loci (QTLs), a genomic strategy, has successfully yielded a large number of hypertension-related QTLs. As a stand-alone technology, however, it has generally failed to identify the definitive set of genes involved in the pathogenesis of hypertension as well as other complex diseases. One of the reasons may be that QTLs generally span large chromosomal segments that incorporate a large number of genes. The major difficulty lies in the ability to reduce the number of genes within the QTLs to those few that are directly involved in the pathogenesis of the disease. To overcome this problem, other biotechnological strategies have been tried. The most commonly used approach so far has been the construction of congenic strains, aiming to reduce the chromosomal span of the QTLs and thereby decrease the number of potential candidate genes within them. This strategy appears to have been successful in reducing the span of QTLs to the single cM range and below. The process of generating congenic strains, however, is lengthy, laborious, and expensive, and the use of congenics is not without pitfalls. High-throughput differential gene expression profiling, a transcriptomic approach, is another strategy that has been widely applied over the past decade in the search for the genetic basis of complex diseases such as hypertension. As in the case of genetic mapping, however, investigators have largely met with failure when applying this technology as a stand-alone approach, the most likely reason being that a very large number of genes are differentially expressed in tissues or organs of contrasting populations, many of which do not bear relevance to the disease under study.

An emerging alternative strategy to the search for the genetic basis of complex diseases consists of integrating the
genomic with the transcriptomic approach. This integration is based on the assumption that genes that are both differentially expressed and that map to a disease-related QTL are likely to be involved in the pathophysiology of that disease. This strategy has the advantage that it combines the power embedded in genetic mapping and in differential gene expression profiling. In the present study, we applied the integrated genomic-transcriptomic approach in our ongoing search for the genetic basis of hypertension in the Sabra rat model of salt-susceptibility. We previously detected in the male Sabra rat two QTLs on chromosome 1, SS1a and SS1b. We now report on differential gene expression profiling in which we contrasted gene expression in the kidneys of male SBH/y and SBN/y and on integration of the resulting data with those of genetic mapping. This integrative approach successfully led to the reduction of the number of genes incorporated within the two QTLs from over a thousand to just seven that become novel candidate genes for salt-sensitive hypertension.

Materials and Methods

The Model

The study was performed in the Sabra rat model of salt-sensitive hypertension, which consists of two distinct strains: the salt-sensitive SBH/y rat, which when fed regular diet remains normotensive but when salt loaded becomes hypertensive; and the salt-resistant contrasting SBN/y strain, which remains normotensive, irrespective of diet (Koffolk; Tel Aviv, Israel). Male rats only were used.

The phenotype under investigation was the susceptibility to hypertension when exposed to dietary salt loading. Specifically, we sought the hypertension “sensitivity” and “resistance” susceptibility genes. These genes render the organism sensitive or resistant to salt, much as is observed in humans.

Animals were studied while provided regular rat chow and tap water ad libitum, or during salt loading. Salt loading was achieved by implanting a 25-mg deoxycorticosterone acetate (DOCA) pellet in the back of the neck, achieving sustained levels of the hormone over 28 days, while providing the animals regular rat chow and 1% NaCl in tap water ad libitum. Blood pressure was measured after 4 weeks of salt-loading or regular diet by the tail-cuff method, using the IITC apparatus. We previously validated the of salt-loading or regular diet by the tail-cuff method, using the IITC

Overview of the Research Strategy

Our overall strategy to detect the salt-susceptibility genes was based on integrating genetic mapping (genomics) with differential gene expression profiling (transcriptomics), as shown in Figure 1. In the genomic arm of the project reported earlier, we identified in male rats two salt-susceptibility QTLs on chromosome 1, SS1a and SS1b. The transcriptomic arm of the project, the results of which are presented henceforth, was performed de novo. We subsequently integrated the data obtained from the two approaches.

Genetic Mapping

In our previous study, we used an F2 cross between SBH/y and SBN/y to scan the entire rat genome for linkage between the salt-susceptibility phenotype and microsatellite markers spread throughout the genome. This random genome screening led us to generate genetic maps and detect salt-susceptibility QTLs. We defined and demarcated the chromosomal location of these QTLs using the MAPMAKER/QTL software.

Differential Gene Expression Profiling

We presently studied gene expression in whole kidneys in a 2x2 design by contrasting two groups of animals (SBH/y and SBN/y) under two conditions (regular chow and salt loading). We thus studied four groups altogether, five rats per group. Each animal was studied individually. We determined gene expression at the same time point in which the QTLs were sought by genetic mapping, ie, 4 weeks after initiation of salt loading, at which time point hypertension invariably evolves in SBH/y but not in SBN/y.

We chose to study gene expression in the kidneys, as opposed to other organ systems that may be of relevance to hypertension or salt-susceptibility, based on a large body of evidence implicating the kidney in the pathogenesis of hypertension in general and salt-sensitivity in particular.

For tissue extraction, we rapidly removed the kidneys from ether-anesthetized animals, snap-froze them in liquid nitrogen and stored them at −80°C until RNA extraction. We extracted total RNA from each animal in separate, using Trizol (Gibco). Processing from hereon through to oligonucleotide microarray hybridization was as previously described. In brief, we prepared cDNA from RNA using T7-(dT)24 primer, transcribed in vitro in the presence of two biotinylated ribonucleotides Bio-11-CTP and Bio-16-UTP, resulting in labeled cRNA. We used fragmented cRNA for hybridization onto the microarray. We used the Affymetrix GeneChip Rat Genome U230 Affymetrix rat expression set (RAE230), allowing expression profiling of more than 30,000 transcripts and variants, including more than 28,000 well-substantiated rat genes. We collected the data by a laser scanning technique and analyzed the pixel levels with commercial software (Affymetrix). We calculated for each transcript an absolute expression level. We assessed chip quality and hybridization experiments by methods previously described. We used for individual data point normalization the RMA-Express 0.2 software.

We compared the level of gene expression between the four study groups by two-way ANOVA, looking for strain effect, treatment effect, and the combined effect of strain and treatment. When we detected significant differences in expression within each set (P<0.0001), we performed cluster analysis using the Cluster software (version 2.11), identifying transcripts that were either differentially or commonly regulated in the respective groups.

Integration of Genetic Mapping

With Transcriptomics

In the first step of the integration process, we identified among the differentially expressed genes those that mapped to chromosome 1 within the defined boundaries of QTLs SS1a and SS1b. We obtained the chromosomal position of the differentially expressed genes from several sources: Affymetrix GeneChip NetAffx Analysis Center database analysis tools (https://www. affymetrix.com/analysis/netaffx/quickquery.affx), Rat Genome Database (RGD) (http://www.rgd.mcw.edu/), NCBI Rat Genome Database (RGD) (http://www.ncbi.nlm.nih.gov/Genomes/Rattus_norvegicus/) and the literature.
In the second step of integration, we determined which of the genes fulfilled two criteria: (1) Differential expression (two-way ANOVA) with a statistical threshold of \( P<0.0001 \) and (2) mapping within the defined boundaries of QTLs \( SS1a \) and \( SS1b \). Those genes that satisfied these two criteria were identified and labeled as highly likely candidate genes for salt-sensitive hypertension and/or salt-susceptibility.

Validation of Microarray Gene Expression Data
To validate differential levels of mRNA abundance detected on the microarray and to determine whether parental strain differences in gene expression could be due to interstrain DNA sequence variants that affect probe binding, we sequenced the target region from select transcripts and assessed mRNA abundance by quantitative RT-PCR (TaqMan). We compared mRNA levels in kidneys under basal conditions and after salt loading. We reverse-transcribed DNA-free total RNA (2g) with oligo(dT) primers (Gibco-BRL), Superscript II reverse transcriptase (Gibco-BRL), and dNTP (Boehringer Mannheim) in 40-uL reaction buffer (Bibco BRL). We designed primers and probes with the Primer Express 1.0 program (PE Applied Biosystems). For TaqMan analysis, we used an Applied Biosystems 7700 system (Perkin Elmer). We normalized expression levels to 18S rRNA expression, using the 2^{-ACT} method.

Results

Linkage Analysis and QTLs for Salt Susceptibility
We previously identified in male rats two QTLs on chromosome 1: \( SS1a \) demarcated by the microsatellite markers \( D1Mkh2 \) and \( D1Mit11 \) (span 43.1 cm) and \( SS1b \) demarcated by \( D1Mit11 \) and \( D1Mit4 \) (span 18 cm).\(^4\) The location of these two adjacent QTLs on chromosome 1 is shown in Figure 2.

Differential Gene Expression Profiling

Study Groups
In the present study, we investigated the differential expression of genes in the kidneys of four groups of animals: hypertensive salt-loaded SBH/y (BP 209±2 mm Hg), normotensive SBH/y provided normal chow and tap water (BP 141±1 mm Hg), normotensive salt-loaded SBN/y (BP 127±2 mm Hg), and normotensive SBN/y provided normal chow and tap water (BP 127±1 mm Hg). There was very little phenotypic variation in terms of blood pressure within each group.

Differentially Expressed Genes
Of the total number of transcripts on the Affymetrix RA230 microarray, 15,442 were expressed in the kidney of SBH/y or SBN/y among any of the four groups studied. The dataset has been submitted to the European Bioinformatics Institute (EMBL-EBI) database repository, accession no. E-MEXP-157. Among the expressed genes, differential expression between any of the groups was observed in a number of genes that varied by the level of statistical significance. Taking \( P<0.0001 \) as the cut-off level, the number of differentially expressed transcripts was 2470.

Biological Relevance and Cluster Analysis
We addressed the relevance of the differentially expressed genes to the effects of strain differences, salt-loading and hypertension. Using the two-way ANOVA and a cluster analysis design, we identified nine clusters, seven of which are shown in Table 1 and Figure 3. Two of these clusters (A-I and A-II) were directly associated with strain differences, two (B-III and B-IV) with the effect of salt-loading per se, two with salt-sensitivity and hypertension (C-V and C-VII), and one with salt-resistance (C-IX). Two additional clusters (C-VI and C-VIII, not shown in Table 1) were identified when contrasting SBH/y (SL) and SBN/y (RD) with SBH/y (RD) and SBN/y (SL). The biological significance of these latter clusters is at present unclear.

Differential Expression by Clusters

Cluster V
Of 494 differentially expressed transcripts (upregulated in salt-loaded SBH/y compared with all other groups), we found a statistically significant interaction of strain and treatment (two-way ANOVA, \( P<0.0001 \)) in 156. As a clear interaction can be demonstrated between the allele status and salt-loading, these genes are most likely to be relevant to salt-sensitivity and/or hypertension.

Cluster VII
Of 245 differentially expressed transcripts (downregulated in salt-loaded SBH/y compared with all other groups), only 36 met the statistical threshold criterion of \( P<0.0001 \) for the interaction of strain and treatment effect (two-way ANOVA). These transcripts are most likely to be relevant to salt-sensitivity and/or hypertension.

Cluster IX
Of 20 differentially expressed transcripts (upregulated in salt-loaded SBN/y compared with all other groups), none met the stringency criterion of \( P<0.0001 \) for the interaction of strain and treatment effect (two-way ANOVA). This analysis did not detect, therefore, transcripts of relevance to salt-resistance.

Chromosomal Localization of Clusters
The chromosomal localization of the differentially expressed transcripts from clusters V and VII is shown in Table 2. Fourteen transcripts from cluster V and four from cluster VII mapped to rat chromosome 1.

Integration of Genetic Mapping With Differential Gene Expression Profiling

Identification of Genes Within QTLs \( SS1a \) and \( SS1b \)
The total number of genes annotated thus far to rat chromosome 1 is 2933 (NCBI, http://www.ncbi.nlm.nih.gov). Of these, 1102 genes mapped to the interval defined by QTLs \( SS1a \) and \( SS1b \), with the upper border delimited by \( D1Rat18/ /Tctex1 \) (1q11/1p11) and the lower border by \( D1Rat272/Rat38 \) (1q31/1q32).

Identification of Genes Within Clusters V and VII That Map to QTLs \( SS1a \) and \( SS1b \)
We selected among the genes that were differentially expressed between the various groups, that reflected the strain-
salt-loading interaction (C-V, C-VII), and that met the $P<0.0001$ significance level in the two-way ANOVA analysis—those that mapped to QTLs SS1a and SS1b. A total of eight genes were thus identified, seven within cluster C-V and one within cluster C-VII, all bearing biological relevance to salt-susceptibility and hypertension. The list of genes that were excluded is provided as the online data supplement available at http://circres.ahajournals.org.

Seven of the eight genes were overexpressed in SBH/y compared with all other groups and one was underexpressed in SBH/y (Table 3). Among the overexpressed genes, the level of differential expression ranged from 109% to 179%.

**Confirmation of Candidate Genes**

In a separate experiment, we hybridized the same RNA samples that were used in the RAE230 study on U34A Affymetrix GeneChips. Three of the eight transcripts that stood out as relevant to salt sensitivity and hypertension using the UAE230 Chipset were also represented on the U34A expression set. The expression abundance of these transcripts and their level of differential expression using the U34A expression set were not different from those obtained using the UAE230 set (Figure 4), thus verifying and confirming our findings in these representative samples in two separate sets of microarray experiments.
Validation of Candidate Genes by RT-PCR
We sequenced the target region from the eight transcripts of relevance. We further studied mRNA abundance of these transcripts in parental strains by quantitative RT-PCR (TaqMan). This analysis resulted in 16 comparisons. Significant sequence differences that could affect probe binding were not found for any of the transcripts examined. Strong concordance of fold change was seen when the microarray results were compared with the results of the RT-PCR for 7 transcripts (Figure 5). A discrepancy between microarray and Taqman results was observed for one transcript, U2AF-like, in SBH/y animals, which led to the exclusion of that transcript. This left us with 7 validated transcripts, TcTex1, Myadm, Lisch7, Axl-like, Fah, PRC1-like, and Serpinh1.

Discussion
In the current investigation, we effectively combined the conventional genomic linkage approach that poses the question “where is the culprit gene?” with the gene expression transcriptomic approach to answer “which is the culprit gene?” Our major significant finding was that by integrating the algorithm “where is the culprit gene?” with the gene expression transcriptional approach that poses the question “which is the culprit gene?” Our major significant finding was that by integrating the two classical strategies, we were able to rapidly reduce the number of candidate genes within the boundaries of our QTLs on chromosome 1 from 1102 to seven validated novel candidate genes for hypertension.

What is known about these novel candidate genes in the context of hypertension? None of the seven genes had been previously directly implicated in the pathogenesis of hypertension. Yet three of these genes, Serpinh1, TcTex1, and Axl-like, can be linked indirectly to hypertension by virtue of their similarity to or belonging to a family of genes that are expected to shed further light on the role of these genes in relation to hypertension.

Additional experiments related to the function of the seven genes, such as cardiovascular phenotyping of knockout mice and of transgenic rat strains, will be required to investigate whether the observed changes in gene expression reflect the primary pathogenic mechanisms we are seeking (cause), as opposed to secondary phenomena (effect). The known identity of the human homologues of these genes should allow us to perform in parallel association studies directly in humans, which are expected to shed further light on the role of these genes in the pathogenesis of hypertension in humans.

The significance of this study lies, however, not only in the detection of new candidate genes for hypertension, but also in the algorithm that led to their detection. The evolution of the algorithm integrating genomics with transcriptomics deserves further comment. Much of the forefront research into the genetic basis of complex diseases, such as hypertension, had been based during the past decade on either genomics or transcriptomics. The expectations of the scientific community that these technologies would rapidly yield the genes involved in such diseases have, in large, not been met. Although a large number of QTLs have been identified and a plethora of genes have been reported to be differentially expressed, neither approach has successfully yielded defini-
tive culprit genes for most complex diseases, including hypertension. Furthermore, investigators have recently come to the realization that it is likely that not a few genes with major effects are likely to be involved but rather an assembly of multiple genes, each with a small effect, which together make up the biological maze that leads to complex diseases. These considerations led Pravenec and coworkers to put forward an alternative conceptual approach that seeks to integrate state of the art genetic technologies and thereby maximize their yield, specifically proposing to combine genetic strategies with genome-wide expression profiling. Adopting the general concept, we applied in the current study a somewhat modified integrative approach. Whereas Pravenec and coworkers proposed integrating genetic/linkage analysis of gene expression levels with genetic/linkage analysis of physiological phenotypes, we used in the present investigation an approach that targeted rather higher priority candidate genes if they (1) were differentially expressed between the parental strains, (2) showed a statistically significant interaction between allele status and salt-loading, and (3) also

Figure 3. Subcluster image. RD indicates regular diet; SL, salt-loading.
mapped within a region linked to the regulation of blood pressure.

We realize that our approach has weaknesses. What are the limitations of the integrated genomic-transcriptomic strategy? The integrative approach necessarily incorporates the problems and limitations inherent in genetic mapping and QTL detection on one hand and of differential gene expression profiling on the other. The limitations of genetic mapping and QTL detection have been previously discussed in depth.\textsuperscript{21} Relating to the genomic arm of our project, we detected in male rats QTLs on chromosome 1 only. The possibility that these QTLs are false-positives is very low, in view of the results of our studies using consomic and congenic strains.\textsuperscript{6} The likelihood that other QTLs are present on additional chromosomes, although they were not detected in our study by linkage analysis (false-negatives), cannot be excluded unless consomic strains are constructed for each chromosome in separate and tested individually. We are engaged in addressing this problem but at this time we cannot rule out with certainty participation of other QTLs in salt-susceptibility or hypertension. Among the limitations of differential gene expression profiling is the key assumption that the disease-causing genes must be expressed in the tissue sampled and that the sampled tissue in which expression is studied must be involved in the pathophysiology of the disease. In the current study, we chose to study gene expression in the kidneys, as the likelihood that this organ is involved in the pathogenesis of salt-sensitive hypertension is high indeed.\textsuperscript{7–9,22–24} The assumption that a gene involved in the pathogenesis of a complex disease must be expressed is reasonable, although not foolproof. There could be false-negatives. One example is that a gene may be expressed only in the developmental stages of a disease, but is no longer expressed once disease is established, thus yielding a false-negative if expression is studied at that stage. Another example is that the level of gene expression is low and below the detectable level of the system (very low abundance). A third example are transcriptional and posttranscriptional changes of importance to the pathogenesis of the disease that would go undetected by differential gene expression profiling. A fourth example is at the posttranslational level, whereby a variant could affect protein function in the absence of any effects on gene expression. There is also the possibility of a false-positive, for example, if a gene is differentially expressed but the difference in the level of expression does not necessarily reflect disease-relevant mechanisms. Another

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\(P<0.0001.\)

TABLE 3. Identity of the Salt-Sensitivity and Hypertension-Related Candidate Genes Within QTLs SS1a and SS1b on Chromosome 1

<table>
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<tr>
<th>Symbol</th>
<th>Cluster</th>
<th>Physical Location</th>
<th>QTL Localization</th>
<th>Gene/EST Description</th>
<th>Human Homologue</th>
<th>SBH/y SL vs SBH/y RD</th>
<th>SBH/y SL vs SBN/y SL</th>
<th>SBH/y SL vs SBN/y RD</th>
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<tr>
<td>Tctex1</td>
<td>C-V</td>
<td>1p11</td>
<td>SS1a</td>
<td>t-complex testis expressed 1</td>
<td>TCTEX1 6q25.2–25.3</td>
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<td>U2AF-like</td>
<td>C-V</td>
<td>1q12</td>
<td>SS1a</td>
<td>Similar to splicing factor U2AF large chain, human</td>
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<td>Myadm</td>
<td>C-V</td>
<td>1q13</td>
<td>SS1a</td>
<td>Human myeloid-associated differentiation factor</td>
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<td>1q21</td>
<td>SS1a</td>
<td>Liver-specific bHLH-Zip transcription factor 7</td>
<td>LISCH7 19q13.12</td>
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<td>SS1a</td>
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<td>SS1b</td>
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<td>Similar to protein regulator of cytokinesis 1</td>
<td>PRC1 15q26.1</td>
<td>1.32</td>
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<td>Serpinh1</td>
<td>C-V</td>
<td>1q32</td>
<td>SS1b</td>
<td>Serine (or cysteine) proteinase inhibitor, clade H, member 1</td>
<td>SERPINH1 11q13.5</td>
<td>1.77</td>
<td>1.57</td>
<td>1.60</td>
</tr>
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</table>
limitation of differential gene expression profiling is in the use of an arbitrary statistical stringency that led in the final analysis to a small number of transcripts. As by definition this statistical stringency was arbitrary, we cannot rule out false-negatives, i.e., that additional transcripts of relevance that would have met lower stringency criteria were omitted. Finally, there is a limitation of the tools used in the analysis of the expression data. In our case, our mode of analysis did not allow us to differentiate between hypertension and salt-susceptibility–related genes. We were thus unable to separate in this experimental model of salt-sensitive hypertension the transcripts related to either of these two distinct phenotypes which, in the hypertension-prone SBH/y strain, are phenotypically closely associated.

Has the integrated genomic-transcriptomic approach rendered the consomic-congenic strategy obsolete? By no means. We must recognize that both approaches are valid and in some ways complementary. In the context of the search for the genetic basis of complex diseases such as hypertension, consomic and congenics remains of ultimate importance, primarily and foremost in confirming the validity of the QTLs. Congenic strains will become irreplaceable when the issue of gene-to-gene interaction is addressed. It is also highly likely that congenic strains will be continued to be used to narrow QTLs, although additional alternative strategies are evolving, one being advanced intercrossed lines. Moreover, congenics also have a role in the integrated approach. Genes that are differentially expressed between congenic strains and the parental contrasting background and that fall within the QTL boundaries become of direct relevance. By narrowing the span of the QTLs, congenic strains can also improve the yield of the integrated approach by diminishing further the number of candidate genes.

In summary, by combining genetic mapping with differential gene expression profiling, we were able to narrow down the list of candidates genes for salt-susceptibility and hypertension in the rat to seven genes on chromosome 1. Even though the identity of the seven candidate genes has been thereby established and some of them have already been related to hypertension, elucidating their function and role in the pathogenesis of hypertension remains a daunting task, well beyond the scope of the present study. Nonetheless, investigating seven genes and their interaction with each other, as opposed to 1102 genes, is a much more feasible task. This study thus bring us one step closer to the better understanding of the relationship between hypertension and salt-susceptibility and the formidable task of identifying and elucidating how the “not so few” genes that form the genetic basis of hypertension work together to cause this disease entity in humans.

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References


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