

New Target Regions for Human Hypertension via Comparative Genomics

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Models of human disease have long been used to understand the basic pathophysiology of disease and to facilitate the discovery of new therapeutics. However, as long as models have been used there have been debates about the utility of these models and their ability to mimic clinical disease at the phenotypic level. The application of genetic studies to both humans and model systems allows for a new paradigm, whereby a novel comparative genomics strategy combined with phenotypic correlates can be used to bridge between clinical relevance and model utility. This study presents a comparative genomic map for “candidate hypertension loci in humans” based on translating QTLs between rat and human, predicting 26 chromosomal regions in the human genome that are very likely to harbor hypertension genes. The predictive power appears robust, as several of these regions have also been implicated in mouse, suggesting that these regions represent primary targets for the development of SNPs for linkage disequilibrium testing in humans and/or provide a means to select specific models for additional functional studies and the development of new therapeutics.

Genetic studies of multifactorial disorders such as hypertension in human populations remain challenging because of multiplicity of genes underlying complex phenotypes, the modest nature of gene effects, and the inevitable heterogeneity of the patient population. Because of the limited success in identifying genes involved in complex traits using linkage studies, map-based association studies and linkage disequilibrium tests have gained momentum as novel approaches, supported by the rapid development of “third generation” markers based on single-nucleotide polymorphisms (SNPs) (Wang et al. 1998; Marshal 1999). These studies require a very high density of genetic markers and sophisticated statistical tools to analyze large marker sets (Lander 1996; Collins et al. 1997; Kruglyak 1997). Once a region is established to harbor a major disease gene, there is potential for advanced “finished” sequencing of these regions (Collins et al. 1998). The huge human and economic cost of hypertension warrants an accelerated discovery pathway for SNP development and advance finished sequencing of regions containing the disease genes. However, little is known about the genetic basis of human hypertension or any other multifactorial disorder (e.g., diabetes, myocardial

infarction, psychiatric disease), limiting these novel strategies until a high-density SNP map is available.

Studies on the genetic basis of hypertension have discovered multiple quantitative trait loci (QTLs) predominantly in rat models (Hamet et al. 1998) and encouraged studies in human populations using the candidate gene approach (Jeunemaitre et al. 1992a,b; Casari et al. 1995). However, with the exception of rare monogenic forms (Shimkets et al. 1994; Simon et al. 1996a,b), limited progress has been made in the identification of underlying genetic factors of essential hypertension and case/control studies using the candidate gene approach have yielded conflicting results (Jeunemaitre et al. 1992a,b; Harrap et al. 1993; Iwai et al. 1994; Casari et al. 1995; Kato et al. 1998; Brand et al. 1998; Niu et al. 1998). A recent study in the mouse has also identified QTLs within the mouse genome contributing to hypertension (Wright et al. 1999). Therefore, we developed a novel comparative genomics strategy for using QTLs from various rat models for genetic hypertension to prioritize regions of the human genome for focused SNP discovery and linkage disequilibrium testing. A sufficient number of high-quality genetic studies conducted in human and mouse provided a means to validate our strategy. In addition to prioritized regions for high-density genotyping in humans using SNPs, the data presented here

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Table 1. Hypertension-Related QTLs Identified in the Progenies of Genetically Hypertensive Rats and Syntenic Chromosomal Regions in Human Genome Based on Cytogenetics

QTL #	QTL cluster	TRAIT	CROSS	LOD-SCORE	INTERVAL	distance (cM)	SYNTENIC REGIONS IN HUMAN
1	1	max DBP	SHR X WKY	4.82	D1MGH7-D1MIT18	60.5cM	15q11.2, 11q14.3-11q14.1, 11p15.4-11p15.1, 16p12.2-16p11.2, 10q25.3-10q24.3
2		max SBP	SHR X WKY	3.542			
3		max MAP	SHR X WKY	4.778			
4		DBP	SHR X WKY	4.884			
5		SBP	SHR X WKY	2.55			
6		MAP	SHR X WKY	4.503			
7		MAP	SHR X DRY	2.71			
8		SBP	FHH X ACI	3.79			
9		PP	LH X LN	2.599			
10		hs basal map	SS X BN	3.184			
11		day2 MAP	SS X BN	3.27			
12		Baseline BP	SHR X BN	2.637			
13	2	SBP	LH X LN	4.24	D2MGH2-CPB	32.8cM	5p14-5q11-q12, 3q21-q26.3, 4q25-q28,
14		DBP	LH X LN	3.717			
15		MAP	LH X LN	4.049			
16		MAPS	SHR X BN	2.882			
17		SBPS	SHR X BN	2.912			
18		salt PP	SHR X BN	3.724			
19		salt PP	SHR X BN	2.91			
20		3	delta SBP	SHR X WKY			
21	tail BP		GH X BN	3.587			
22	la BP		GH X BN	2.58			
23	delta_wake_m_day2am_SBP		SS X BN	3.66			
24	MAPS		SHR X BN	4.128			
25	DBPS		SHR X BN	3.074			
26	salt PP		SHR X BN	2.91			
27	4	delta_hs_m_la_MAP	SS X BN	3	D3MGH16-D3MIT8	36.4cM	9q34.3-9q32-q33.1, 2q14-q23
28		delta SBP	SHR X WKY	2.575			
29		delta MAP	SHR X WKY	2.857			
30	5	delta MAP	SHR X WKY	2.787	D4MIT23-D4MGH16	27cM	7q35-7q21, 7p14-p15, 2p12
31		delta PP%	SHR X WKY	3.596			
32		delta SBP%	SHR X WKY	2.927			
33		delta MAP%	SHR X WKY	4.183			
34		Captopril	SHR X BN	2.619			
35		MAPS	SHR X BN	4.019			
36		SBPS	SHR X BN	4.1			
37		DBPS	SHR X BN	3.582			
38		DBP	SHR X WKY	2.698			
39	6	delta MAP	SHR X BN	2.611	D5MIT5-D5MGH13	35.9cM	1p32-p31 - 1p35-p31.3
40		delta MAP%	SHR X BN	2.836			
41		delta SBP	SHR X BN	3.877			
42		delta SBP%	SHR X BN	4.125			
43	7	tail BP	GH X BN	4.63	D6MIT12-D6MIT3	39cM	NOT CONVERTIBLE
44		la BP	GH X BN	6.807			
45	8	MAPS	SHR X BN	2.594	D8MIT7-ACAA	54cM	11q23-11q22.1, 15q23-q24 -15q21, 3p11-3p21.3
46		salt PP	SHR X BN	3.482			
47		SBPS	SHR X BN	3.204			
48		delta_hs_m_la_SBP	SS X BN	3.398			
49	9	Hexamethonium	SHR X BN	2.938	D10MIT5-BAND3A	59.4cM	5q31-q33, 17p11.2-17q12-q21
50	10	Baseline BP	SHR X BN	3.19	D11MIT1-D11MIT6	20cM	NOT CONVERTIBLE
51	11	delta_wake_m_day2am_MAP	SS X BN	3.398	D13MGH19-D13MGH10	46.1cM	2q14-q11, 1q32-1q21-q23
52		SBP	LH X LN	5.044			
53		DBP	LH X LN	6.922			
54		MAP	LH X LN	6.466			
55	12	delta_wake_m_day2am_SBP	SS X BN	3.53	D15MGH5-D15MGH8	27.7cM	13q14.3-13q22
56	13	MAPS	SHR X BN	3.325	D16MIT2-D16MGH1	18.3cM	10q23.3, 18p13.1, 13q34, 8p12-8p23
57		SBPS	SHR X BN	3.654			
58		DBPS	SHR X BN	2.981			
59	14	SBP	FHH X ACI	3.38	D17MIT2-D17MIT7	24.3cM	5q32, 7p14-p15
60	15	delta_hs_m_la_SBP	SS X BN	3.843	D18MGH4-D18MGH2	28.6cM	18q21-18q23, 5q21-q31
61		delta_hs_m_la_MAP	SS X BN	4.61			
62		hs basal MAP	SS X BN	3.88			
63		hs basal SBP	SS X BN	3.07			
64		hs active MAP	SS X BN	4.38			
65		Day1 MAP	SS X BN	3.58			
66		Day2 MAP	SS X BN	4.41			
67		tail BP	GH X BN	4.089			

Data shown represent the 99% C.I. for each QTL trait. The total genetic distance covered by QTLs in the rat was 567.6 cM based on a drop of 1.6 lod units from the peak. For detailed phenotype description and abbreviation please see Table 3. QTLs 10, 11, 23, 27, 55, 61, 62, 63, 64, 65, and 66 were mapped nonparametrically using the 'np scan' option available in MAPMAKER/QTL (v.1.9).

also provide investigators with valuable phenotype and mapping information on blood pressure phenotypes that may be beneficial for positional cloning efforts and drug discovery, for example, using congenic

animals. Here, we report a total of 67 QTLs for 39 blood pressure traits in the progenies of seven F₂ rat intercrosses for genetic hypertension on the basis of which 26 homologous regions are prioritized target regions

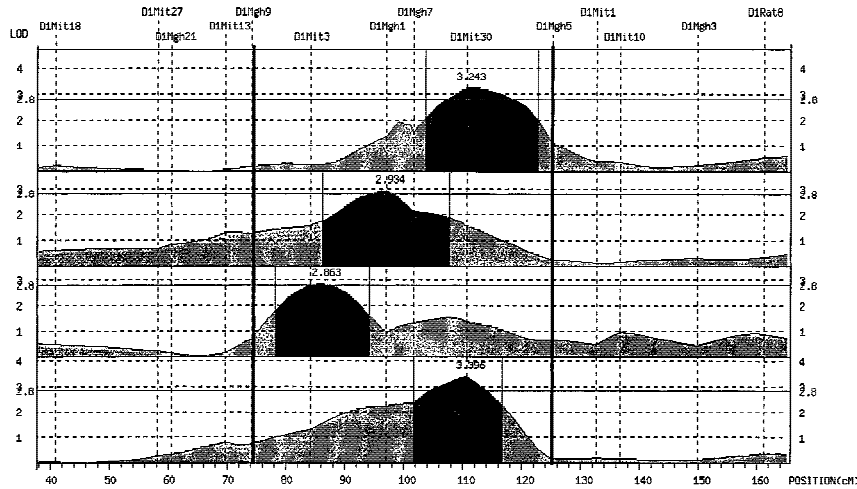


Figure 1 Demonstration of QTL clustering. The dark-shaded area of the QTL profile represents the 99% C.I. of each QTL. The dark vertical lines indicate the boundary of the QTL cluster, which is defined by the nearest two markers flanking the combined 99% C.I., in this example D1Mgh9 and D1Mgh5.

for human genetic studies. Importantly, when validating our strategy by comparing our results with those from recent genome-wide scans in human populations (Julier et al. 1997; Mansfield et al. 1997; Krushkal et al. 1998), five out of the six known QTLs for human hypertension were correctly predicted based on our studies using rat models. Additionally, seven QTLs for blood pressure identified recently in the mouse (Wright et al. 1999) fall within four intervals identified in our rat studies, one of which has been confirmed in humans (Krushkal et al. 1999). Therefore, the predicted human regions represent genetically validated targets for linkage disequilibrium testing using SNPs.

RESULTS

Using the total genome scan approach, we identified a total of 67 QTLs for a total of 39 blood pressure-related traits (see Methods for details) in seven F_2 progenies (Table 1) derived from different genetically hyperten-

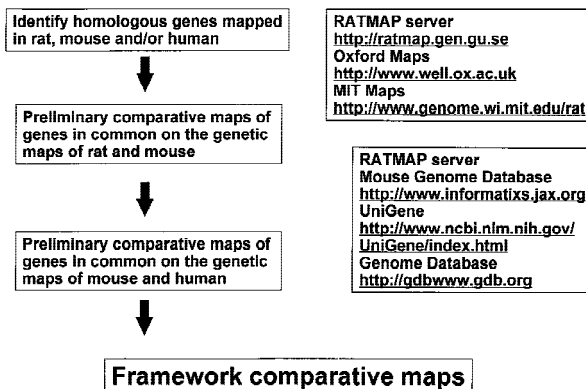


Figure 2 Flow chart showing the algorithm for constructing framework comparative maps between rat, mouse, and human genomes based on publicly available databases.

sive rat strains. These 67 QTLs were integrated on an integrated map and clustered in 15 independent genomic regions. An example for construction of a cluster is shown in Figure 1. Table 1 shows the location of the QTLs identified, including the genetic markers flanking the combined 99% confidence interval (C.I.), genetic distance covered, and lod scores for the given trait in the respective rat intercross, in total, ~500 cM, ~30% of the rat genome. Comparative maps were constructed based on conserved genomic regions and evolutionary breakpoints between rat, mouse, and human genomes (Fig. 2) using homologous genes as anchors.

Based on these comparative maps, the 67 QTLs identified in the rat were translated to 26 chromosomal segments that are located on 16 autosomes in the human genome (Fig. 3; Table 2). Of these segments, 20 come from 8 distinct QTL clusters (regions of overlapping QTLs) identified on rat chromosomes 1, 2, 3, 4, 8, 13, and 18 (Table 1) and are designated as first priority regions covering ~22.5% of the human autosomal genome. These clusters represent areas where multiple blood pressure-related traits were linked in multiple crosses and, therefore, are likely to be essential regions. Five regions predicted based on our rat studies (1q, 2p13, 5q31, 15q22, and 17q) were recently identified in genome-wide scans for human hypertension (Julier et al. 1997; Mansfield et al. 1997; Krushkal et al. 1998) (Fig. 3, flagged with a star). Table 2 provides the 26 predicted chromosomal segments of the human genome based on cytogenetics as well as a list of the 412 genetic markers currently available based on GeneMap98 at the National Center for Biotechnology Information (NCBI). This table also lists some of the obvious candidate genes within each interval.

The 26 regions in the human genome represent ~30% of the genome, suggesting that the overlap between the rat and human data could be a chance occurrence. Estimates on the bin size of QTL regions in rat and human genomes, respectively, showed that our predictions of target regions in the human genome were not due to chance (z -test, $P < 0.001$; see Methods for details). Furthermore, five out of six regions have been identified in genome scans in humans, with two of the predicted regions (5q and 17q) confirmed in two independent studies. Moreover, recent studies in the mouse have identified several QTLs that contribute to high blood pressure in this model. The mouse QTLs are syntenic with the QTLs on rat chromosomes 2, 3, 8, and 18 and on predicted regions on human chromo-

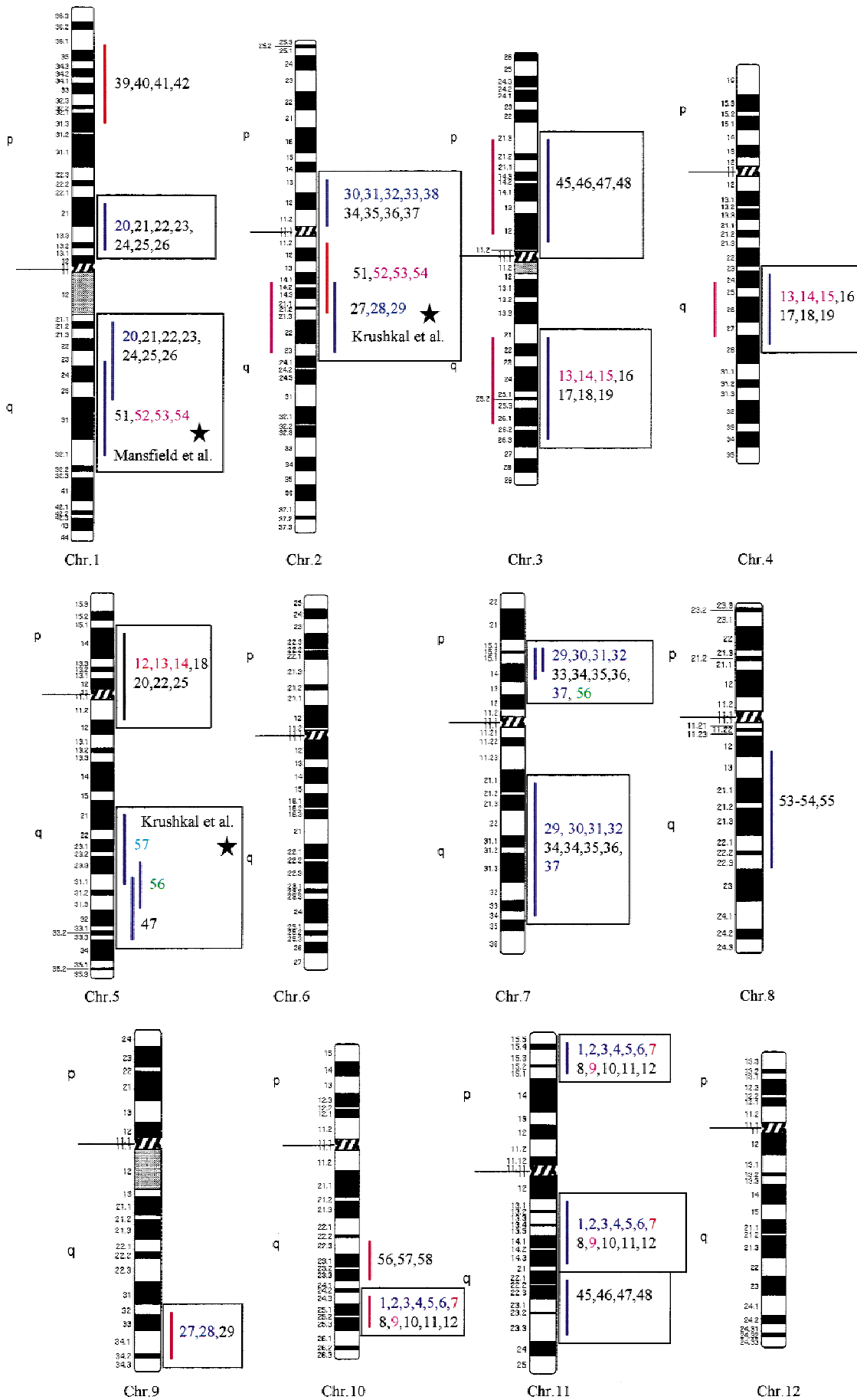


Figure 3 (See facing page for legend.)

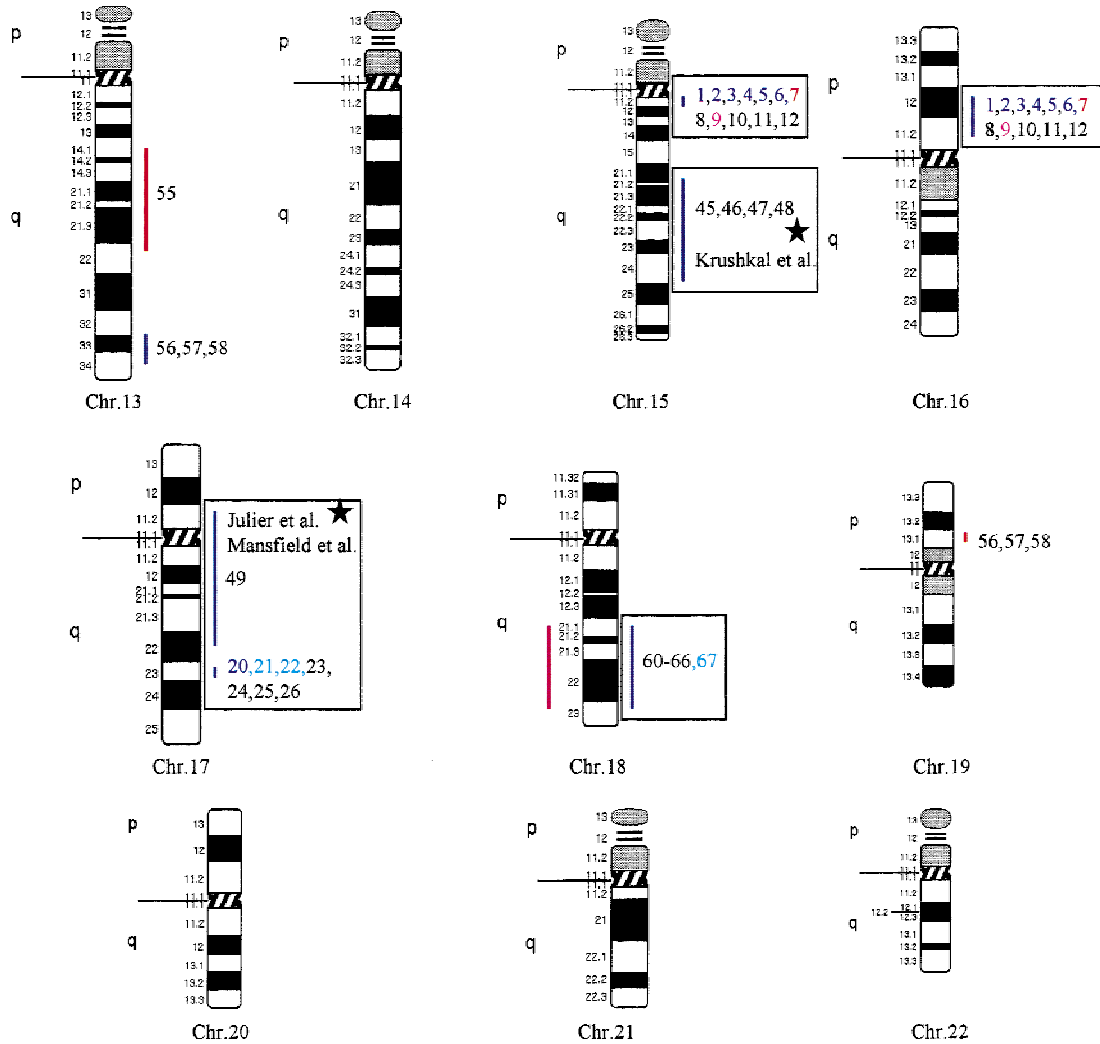


Figure 3 Human chromosomal regions implicated in hypertension based on rat-human comparative maps for 67 QTLs identified in seven different progenies from genetically hypertensive rats. Distribution of the 26 predicted genomic regions in the human genome located on 16 different autosomes based on cytogenetics. Human chromosomes 6, 12, 14, 20, 21, and 22 do not contain positional candidate loci predicted by the rat studies, and the sex chromosomes were not evaluated in this study. Regions of the genome in which multiple blood pressure-related phenotypes cluster from more than one cross are designated first priority regions. There are 20 of 26 regions that fall into this category (boxed regions). We predict six “second priority regions” based on QTLs identified in a single rat cross that may represent either areas of interest for a distinct subtype of hypertension or areas harboring modifier genes for blood pressure (nonboxed regions). Confidence level for placement of syntenic regions designated by vertical bars: (Red) Highest, (blue) high, (black) moderate. Numbers: (Black) SHR × BN, (purple) SS/MCW × BN, (blue) SHR × WKY, (red) SHR × DRY, (dark green) ACI × FHH, (light green) GH × BN, (magenta) LH × LN. Mouse syntenic QTLs are represented by the vertical bars to the left of the human ideograms. (Star) Genomic region reported with significant or suggestive linkage in genome scans for hypertension in humans.

somes 2q14–q23, 3p11–3p21.3, 3q21–q26.6, 4q25–q28, 5p14–q12, and 18q21–q23, further suggesting conserved regions contributing to blood pressure regulation (Fig. 3).

DISCUSSION

The recent improvement of analytical techniques in molecular genetics has provided powerful tools to identify the genes involved in heritable diseases. However, polygenic disorders in humans remain challenging. Power estimates suggest that identification of

genes responsible for common polygenic disorders will most likely come from association studies (Risch and Merikangas 1996). Unfortunately, association studies require specific candidate genes or closely spaced markers (at an average spacing of at most 150 kb) to be tested. Given that the third generation map of the human genome is not immediately available, there is a need to select candidate regions for early SNP development. Our strategy is to use animal models. Prior to this publication, investigators have used QTLs in the rat to select candidate genes for testing in human populations. For example, we and others have reported

Table 2. Predicted Chromosomal Segments in the Human Genome Based on Synteny to Blood Pressure QTLs in the Rat

Rat QTL	INTERVAL	GENETIC MARKERS IN INTERVAL	DISTANCE (cM)	CANDIDATE GENES
1	11q14.3-q14.1 11p15.2-p15.5 16p11-p12.2 10q26-q26.3	D11S1354, D11S1911, D11S911, D11S4172 D11S1307, D11S921, D11S1780, D11S1318, D11S909, D11S922 D16S412, D16S417, D16S3093, D16S409 D10S209, D10S587, D10S217, D10S210, D10S590, D10S555, D10S1651	10.4cM 24.9cM 14.6cM 31cM	SA, SCNN1B
2	5p14-5q11 3q21-3q26.3 4q25-4q28	D5S651, D5S634, D5S426, D5S395, D5S2101, D5S1994, D5S455, D5S428, D5S1977, D5S641, D5S626, D5S637 D3S1306, D3S1555, D3S1553, D3S1580, D3S3579, D3S3702, D3S1268 D31258, D3S1614, D3S1564, D3S1548, D3S3715, D3S1601, D3S3642 D4S2945, D4S430, D4S1611, AFM10ydc6, D4S1588, D4S1546	35.6cM 56.9cM 38.5cM	ANG II AT1 receptor, Kininogen, SLC2A2, FGF ET-A receptor, Aldosterone receptor, NPY2R
3	1q22-1p13 17q23.1	D1S514, D1S2636, D1S2344, D1S498, D1S2858, D1S2624, D1S418 D1S2756, D1S2748, D1S250, D1S2881, D1S467, D1S189, D1S2875 D1S453, D1S2865, D1S418, D1S223, D1S719, D1S739, D1S767, D1S2789 D17S794, D17S795, D17S807, D17S942, D17S940	46.2cM 6cM	FGFR2, Annexin, TIMP-3, IL6R, NPR1 IGF2R, SCL16A1, NRAS, NGFB, TGFBR3, CALPONIN3 ECGF1, VCAM1, ADORA3, KCNA3 Dihydropyridine sensitive L-type Ca-channel
4	9q34.3-9q32 2q14-q23	D9S298, D9S1821, D9S1861, D9S1796, D9S1821, D9S159, D9S279, D9S159 D2S298, D2S284, D2S356, D2S321, D2S142, D2S156, D2S376	7.2cM 23.6cM	PTSG1, Tenascin C DPP4, SCN2A1, ATP1G1
5	7q35-7q21 7p14-7p15 2p12	D7S2450, D7S550, D7S661, D7S498, D7S688, D7S2439, D7S483 D7S2462, D7S637, D7S2423, D7S523, D7S655, D7S2554, D7S687 D7S2502, D7S2543, D7S486, D7S622, D7S480, D7S655, D7S643 D7S650, D7S2473, D7S490, D7S686, D7S1873, D7S1874, D7S635 D7S504, D7S2501, D7S530, D7S2519, D7S631, D7S2438, D7S681 D7S509, D7S2468 D7S529, D7S484, D7S516, D7S2496, D7S632, D7S2252, D7683 D2S145, D2S286, D2S388, D2S113	60.1cM 15.6cM 20.2cM	SLC4A2, LQT2, PPP1R3 TAC1R
6	1p31 - 1p35	D1S2741, D1S203, D1S220, D1S2770, D1S2873, D1S885, D1S846 D1S209, D1S246, D1S2886, D1S198, D1S2806, D1S192, D1S501 D1S411, D1S448, D1S219, D1S481, D1S216, D1S500, D1S2618, D1S207 D1S488, D1S2774, D1S2766, D1S2843, D1S417, D1S2732, D1S478 D1S2884, D1S482, D1S2749, D1S449, D1S2693, D1S513, D1S179 D1S231, D1S2661, D1S447, D1S2861	75.1cM	JUN, CYP2J2, AK2, JAK1, LEPR, ECE, CYP4A11, EDN2
7	11q23-11q22.1 15q24 -15q21 3p11-3p21.3	D11S939, D11S924, D11S1347, D11S938, D11S1327, D11S927, D11S1347 D15S124, D15S160, D15S114, D15S889, D15S115, D15S200, D15S152 D3S3606, D3S3554, D3S1587, D3S1292, D3S1615, D3S1549, D3S1576, D3S1316, D3S1609, D3S1260, D3S3727, D3S1561, D3S1611, D3S1260 D3S3582, D3S687, D3S3685, D3S3559, D3S578, D3S1588	14.3cM 6.9cM 98.4cM	ATP1G1, APOC3, HTR3 CYP1A1, CYP1A2, CYP11A, TPM1 PPP2R3, TGFBR2, SCN5A
8	17p11.2-17q21 5q31-5q33	D17S791, D17S794, D17S806, D17S797, D17S1795, D17S809 D17S941, D17S788, D17S790, D17S787, D17S1607, D17S808, D17S1828, D17S786, D17S796, D17S938, D17S807, D17S799, D17S922, D17S921, D17S853, D17S805, D17S842, D17S1800, D17S1680, D17S798, D17S933 D5S471, D5S393, D5S467, D5S622, D5S2059, D5S490, D5S1995, D5S396, D5S2117, D5S479, D5S414, D5S500, D5S470, D5S2077 D5S670, D5S497, D5S2013, D5S2014, D5S638, D5S2033, D5S434	74.4cM 28.2cM	NGFR, MYL4, NOS2, ATP1B2, SLC2A4, MYH3, CHRNB1 IL4, IL5, SPARC, ADRB1
9	1q32-1q21	D1S306, D1S491, D1S2683, D1S510, S1S504, D1S2872, D1S456 D1S249, D1S2735, D1S2727, D1S2891, D1S2622, D1S306, D1S2716 D1S2640, D1S461, D1S466, D1S202, D1S240, D1S2701, D1S196, D1S210 D1S431, D1S445, D1S2658, D1S433, D1S2635, D1S2844, D1S2707, D1S464, D1S426, D1S2676, D1S194, D1S196	62cM	C4BP, ADORA1, MYO6, ATP2B4, IL10, DAF, TNNT2 CACNA1S, TNNT1, PTGS2, LAMC1, SELECTIN, APOA2, ATP1A2
11	2q14-2q11	D2S110, D2S2215, D2S2224, D2S343, D2S2294, D2S383, D2S2339 D2S2271, D2S121, D2S363, D2283, D2S2258	10.3cM	
12	13q14.3-13q22	D13S328, D13S168, D13S153, D13S163, D13S176, D13S172, D13S1280 D13S275, D13S156, D13S162, D13S160	16.9cM	EDNRB, 5HT2-RECEPTOR, STAT5
13	10q23.3-10q11.2 13q34 8p12-8p23	D10S541, D10S564, D10S1765, D10S195, D10S1752, D10S1730, D10S605 D10S1645, D10S206, D10S201, D10S1786, D10S551, D10S532, D10S220 D10S539, D10S568, D10S567, D10S549, D10S207, D10S589, D10S561 D10S599, D10S1646, D10S210, D10S1666, D10S1742, D10S560, D10S537 D10S584, D10S218, D10S188, D10S804, D10S1772 D13S285, D13S293 D8S505, D8S518, D8S1803, D8S1791, D8S515, D8S255, D8S268	32.6cM 4.7cM 5.8cM	GLUD1 ATP4B FGFR1
14	5q32 7p14-p15	D5S504, D5S677, D5S429 D7S528, D7S485, D7S2497, D7S2507	7.1cM 1.7cM	DRD1, SLC17A2
15	18q21-18q23 5q21-5q31	D18S474, D18S64, D18S484, D18S1127, D18S69, D18S1144, D18S1103 D18S560, D18S1091, D18S58, D18S70, D18S1161, D18S1009, D18S465 D18S483, D18S1092 D5S495, D5S492, D5S654, D5S869, D5S433, D5S505, D5D656, D5S436 D5S402, D5S2119	52.4cM 39.4cM	GRL1, FGF1, PDGFRB, EGR1, ADRB2

Genetic markers and candidate genes provided represent markers, as mapped and listed in GeneMap98. Homologous regions are listed in relation to the respective genetic location in the rat, and putative candidate genes were chosen based on MIM-Maps and verified for map position in the syntenic region in mouse/rat using the MGD. Of 67 QTLs, 64 were converted to discrete homologous regions in the human genome; 3 QTLs (QTLs 43, 44, and 50) could not be converted because of incomplete coverage of the rat genetic map or lack of homologous genes being mapped between rat, mouse, and human.

that a QTL identified in the rat harboring the angiotensin converting enzyme (ACE) gene was linked to high blood pressure (Hilbert et al. 1991; Jacob et al. 1991). Quickly following these publications, Jeunemaitre et al. (1992a) reported that ACE was not linked to hypertension in humans. Nonetheless, ACE was tested in many populations with the majority of the studies concluding that the human ACE gene was not linked to hypertension (Staessen et al. 1997). However, other genes in the vicinity of ACE could not be ruled out. Two recent publications reporting that QTLs in the region of ACE were linked to high blood pressure in humans (Julier et al. 1997; Mansfield et al. 1997) suggested that QTLs identified in the rat may be predictive. Therefore, we constructed a comparative genomic map for "candidate hypertension loci in humans" based on translating QTLs between rat and human, and vice versa. As this is a novel strategy, it was fundamental to validate the approach. For validation, we compared our predictions with results from the literature (Julier et al. 1997; Mansfield et al. 1997), the Family Blood Pressure Program (Krushkal et al. 1998, 1999) as well as results from studies in the mouse (Wright et al. 1999). Strikingly, there was tremendous overlap between the QTLs identified in the rat, mouse, and human. The identification of a genomic interval where blood pressure QTLs were identified across human (2q14–q23), mouse (chromosome 2), and rat (chromosome 3) genomes further supports the power of comparative mapping and validates the use of this strategy. As more studies are underway, for example, in the mouse, we expect additional QTLs to be identified that are likely to coincide with blood pressure loci in the syntenic regions of the rat.

We studied seven different rat crosses that represent five of the nine inbred strains of genetically hypertensive rats (Stoll and Jacob 1999) and identified 67 QTLs for 39 blood pressure traits. Many of the loci presented in Table 1 have also been reported by other investigators (Hamet et al. 1998), providing additional evidence that these loci play a major role in high blood pressure in the rat. Interestingly, none of the QTLs in the rat were found in all seven crosses, illustrating the degree of locus heterogeneity (different genes) that exists even in a simplified model. We have also found that age and method of blood pressure measurement affect QTL mapping (data not shown). Because the different genetically hypertensive rats display different etiologies of hypertension, we reasoned that the integration of the results from all crosses would collectively mimic the heterogeneous clinical picture of hypertension and the homologous human region candidates for investigation. The data presented here could be used in human association studies. Such a strategy has been used to investigate sequence variation across two candidate genes, ACE (Keavney et al. 1998; Rieder et al. 1999) and lipoprotein lipase gene

(Nickerson et al. 1998), illustrating the power of using SNPs and haplotype structure (even within a single gene) in the localization and identification of high-risk susceptibility mutations for complex diseases. As a starting point for developing SNPs in the regions implicated, we provide a list of the 412 genetic markers currently available for the 26 regions of the human genome in Table 2. These markers can be used to determine ESTs that are harbored within the interval, which in turn can be used to develop SNPs in coding regions. Furthermore, the limited number of "first priority regions" (representing 22.5% of the autosomal genome) implicated in blood pressure regulation could be rapidly developed and tested in the existing collections of patient populations for essential hypertension. As more genetic studies are completed in humans, we predict that the number of syntenic regions for the blood pressure QTLs will also increase, many in the regions predicted here.

Although the predictive power of comparative mapping can be used to prioritize regions to develop large numbers of SNPs, the most likely important aspect of these data is that they provide investigators with a means to select a model system that shares phenotypic and genomic content with a clinical population. In this regard, developing specific congenics (designer congenics) that share phenotypic and genotypic characteristics would yield a powerful platform for functional studies, especially with respect to the physiology and pharmacology of the cardiovascular system. Furthermore, well-defined genetic models open a wide range of possibilities for the identification of targets and the development of new therapeutics. Finally, the cloning and functional characterization of susceptibility genes for multifactorial diseases will most probably require animal model systems. Here, we illustrated that as comparative maps improve and more biological traits are linked to the genome, it will become increasingly easier to integrate the power of functional studies in animal models into a greater understanding of human diseases and, hopefully, improved therapeutic outcomes.

METHODS

Linkage Analysis of Hypertension-Related Traits in Various Genetically Hypertensive Rat Intercrosses

This study used five different hypertensive rat strains: the spontaneously hypertensive rat (SHR); the Dahl salt-sensitive rat rederived at the Medical College of Wisconsin (SS/MCW); the Lyon hypertensive (LH) rat; the fawn hooded hypertensive rat developed at Erasmus University (FHH/EUR) and the genetically hypertensive rat (GH); and four different normotensive strains, the Brown–Norway (BN), the Lyon normotensive (LN) rat, August, Copenhagen, and Irish (ACI), and Donryu (DRY). Seven sets of F₂ intercross progeny from mating genetically hypertensive and normotensive inbred strains (SHR × WKY, SHR × BN, SS × BN, LH × LN, FHH/EUR × ACI, GH × BN, and SHR × DRY) totaling 1687 animals and 39 blood pressure-related phenotypes were studied. The protocols for each study

and the methods for estimating blood pressure varied between the seven studies; therefore, each estimate of blood pressure is treated as an independent estimate to avoid biasing the data set. The details of the specific experimental protocols used for some of the studies were described previously (Harris et al. 1995; Schork et al. 1995; Brown et al. 1996; Innes et al. 1998) and are summarized in Table 3. For linkage analysis, genomic DNA was extracted from liver and spleen using standard methods (Jacob et al. 1995). Genome-wide scans for all autosomes were performed independently in each cross using between 180 and 250 polymorphic simple sequence length polymorphism (SSLP) markers with an average spacing of 10 cM as described previously (Schork et al. 1995; Brown et al. 1996; Innes et al. 1998). Phenotype distributions were tested for normality using the Kolmogorov-Smirnov (KS) test (Fisher and van Belle 1996) prior to parametric and/or nonparametric linkage analysis using the

MAPMAKER/QTL computer package (v. 1.9) (Lander et al. 1987). Thresholds for the lod scores were established for our cross structure in accordance with Lander and Kruglyak (1995), where a lod score >2.8 was suggestive and a lod score >4.3 was significant for an F₂ intercross. Eighty-two percent (55 out of 67) of the rat QTLs were >2.8. In addition, 12 QTLs with an lod score of >2.5 were included under the premise that they were located in a genomic region that contained at least one more QTL that reached a minimal lod score of 2.8. The 99% C.I. for each QTL in a given cross was determined by calculating the genetic distance based on the drop of 1.6 lod units from the peak. The use of the 1.6 lod unit drop to define the C.I., rather than the traditional lod drop of 1, results in an expansion of the interval by ~25%. However, we believe this more conservative approach is critical to minimize the number of type II errors (missed linkages), when translating between species in a predictive way.

Table 3. Phenotype Designation Key for Mapped QTLs in Progenies from Hypertensive Rat Strains

Rat cross	Phenotype description
Max DBP	SHR × SKY maximal diastolic blood pressure
Max SBP	SHR × WKY maximal systolic blood pressure
Max MAP	SHR × WKY maximal mean arterial blood pressure
DBP	SHR × WKY diastolic blood pressure (baseline)
SBP	SHR × WKY systolic blood pressure (baseline)
MAP	SHR × WKY mean arterial blood pressure (baseline)
MAP	SHR × DRY mean arterial blood pressure
SBP	FHH × ACI systolic blood pressure
PP	LH × LN pulse pressure
Hs basal MAP	SS × BN mean arterial blood pressure, arterial catheter implanted, 4 weeks high salt diet (8%) mean of measurements on 3 consecutive days, basal state—lights on and rat asleep
Day2 MAP	SS × BN mean arterial blood pressure, 4 weeks high salt diet (8%), day 2 measurement
Baseline BP	SHR × BN baseline blood pressure average of 30 min on regular diet
SBP	LH × LN systolic blood pressure
DBP	LH × LN diastolic blood pressure
MAP	LH × LN mean arterial blood pressure
MAPS	SHR × BN mean arterial blood pressure average of 30 min after 14 days high salt diet on 1% NaCl
SBPS	SHR × BN systolic blood pressure average of 30 min after 14 days high salt diet on 1% NaCl
DBPS	SHR × BN diastolic blood pressure average of 30 min after 14 days high salt diet on 1% NaCl
Salt PP	SHR × BN pulse pressure average of 30 min after 14 days high salt diet on 1% NaCl
ΔSBP	SHR × WKY systolic blood pressure, change from baseline after 'startle'
Tail BP	GH × BN blood pressure measured by sphygmomanometer
ia BP	GH × BN intra-arterial blood pressure
Δ_wake_m_day2a m_SBP	SS × BN systolic blood pressure, high salt diet, p.m. active state value minus a.m. basal state
Delta_hs_m_ls_MAP	SS × BN mean arterial pressure, high salt minus low salt, basal state—lights on and rat asleep
ΔMAP	SHR × WKY mean arterial pressure, change from baseline after startle
ΔPP%	SHR × WKY pulse pressure, percent change from baseline after startle
ΔSBP%	SHR × WKY systolic blood pressure, percent change from baseline after startle
ΔMAP%	SHR × WKY mean arterial pressure, percent change from baseline after startle
ΔMAP	SHR × BN delta mean arterial pressure after 14 days (1% NaCl) minus mean arterial pressure on low salt
ΔMAP%	SHR × BN delta mean arterial pressure after 14 days (1% NaCl) minus mean arterial pressure on low salt expressed as percentage
ΔSBP%	SHR × BN delta systolic blood pressure after 14 days (1% NaCl) minus systolic blood pressure on low salt expressed as percentage
ΔSBP	SHR × BN delta systolic blood pressure after 14 days (1% NaCl) minus systolic blood pressure on low salt
Delta_wake_m_day2a m_MAP	SS × BN mean arterial blood pressure, high salt diet, p.m. active state value minus a.m. basal state
Delta_hs_m_ls_SBP	SS × BN systolic blood pressure, high salt minus low salt, basal state—lights on and rat asleep
Hs basal SBP	SS × BN systolic blood pressure, arterial catheter implanted, 4 weeks high salt diet (8%) mean of measurements on three consecutive days, basal state—lights on and rat asleep
Hs active MAP	SS × BN mean arterial pressure, arterial catheter implanted, high salt diet, active state—lights off and rat awake
Day1 MAP	SS × BN mean arterial blood pressure, 4 weeks high salt diet (8%), day 2 measurement
Captopril	SHR × BN mean arterial blood pressure after administration of Captopril
Hexamethonium	SHR × BN mean arterial blood pressure after administration of Captopril and Hexamethonium

Generation of QTL Clusters Among the Various Crosses

Blood pressure-related QTLs (99% C.I.) with a lod score >2.8 lod units identified in the rat progenies were integrated into a map that was constructed based on genetic information of all crosses used. Clusters of QTLs in the same genomic region were defined as the genomic region in which two or more pressure-related QTLs overlapped within their 99% confidence intervals. The boundary for each "QTL cluster" was defined by the two nearest markers flanking the "combined 99% C.I." as opposed to the average 99% C.I. (Fig. 1).

Construction of Comparative Maps Between Rat and Human

Map construction was initiated by building framework maps, which were constructed by identifying genes in evolutionarily conserved genomic regions among mammalian species (human, mouse, and rat) that were mapped in rat and mouse and were listed in at least one database containing rat genome data (<http://ratmap.gen.gu.se>; <http://www.well.ox.ac.uk>). Conserved regions and evolutionary breakpoints between rat and mouse genomes were identified using the Mouse Genome Database (MGD) with the mapped genes serving as anchoring points within the published genetic maps for both species. The order of genes was determined in the mouse using linkage groups available at MGD (<http://www.informatics.jax.org>) identifying conserved regions and evolutionary breakpoints between rat and mouse genomes. This information was used to define regions of conserved gene order and evolutionary breakpoints with the human genome, using mapping information of homologous genes in the human genome available in MGD, The Genome Database (<http://gdbwww.gdb.org>), and the UniGene set at the NCBI (<http://www.ncbi.nlm.nih.gov>). (Fig. 2).

Integration of QTL Clusters onto the Homologous Regions of the Human Genome

Based on the comparative maps between rat, mouse, and human genomes, rat QTL clusters were integrated onto the human genome at three confidence levels. The criteria for placement were as follows: (1) highest confidence level: Both markers flanking the 99% C.I. were gene based and define a region of conserved gene order between rat and human and several additional genes within the interval provide additional confidence. (2) High confidence level: one flanking marker is gene based; the other flanking markers is anonymous but in close proximity (~ 5 cM) of a gene mapped in both species. Several additional markers or genes within the interval agree with the defined conserved region. (3) Moderate confidence level: Flanking markers are anonymous but in the vicinity of mapped genes (~ 5 cM). Additional markers within the interval help to include or exclude genomic regions. The cytogenetic location and, when possible, the respective human genetic markers defining the boundaries for the predicted genomic regions were established based on mapped genes in the Human GeneMap98 at NCBI (<http://www.ncbi.nlm.nih.gov/genemap98>) (Deloukas et al. 1998).

Likelihood to Identify Predicted QTLs in Human Studies

To determine accuracy of QTL prediction across species, various statistical tests were performed to assay for likelihoods that the observed correct predictions of five QTLs identified in human studies were not a chance occurrence. For this, z-tests and χ^2 tests were performed based on the following assumptions: The genomic distance covered by rat QTLs and by pre-

dicted regions was $\sim 30\%$ of the complete autosomal genome. If this hypothesis was correct, we would have expected that two of the six (one-third) confirmed human regions would map to the predicted region by chance. Yet five of six (five-sixths) did. In a more stringent test, the genomic regions were binned based on the observed average genomic distance covered by a QTL (30 cM in the rat) and the average size of the homologous region in the human genome (50 cM). In the rat, 15 out of 50 bins showed a linkage with suggestive/significant lod scores for blood pressure QTLs, whereas in the human genome, 26 out of 60 bins were predicted. A z-test showed a significant difference between the frequencies with a $P < 0.001$, confirming that our observation was not a chance occurrence.

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