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An Extreme-Sib-Pair Genome Scan for Genes Regulating Blood Pressure

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Summary

Hypertension, a risk factor for many cardiovascular, cerebrovascular, and renal diseases, affects one in four Americans, at an annual cost of >$30 billion. Although genetic mutations have been identified in rare forms of hypertension, including Liddle syndrome and glucocorticoid-remediable aldosteronism, the abundance of plausible candidate genes and potential environmental risk factors has complicated the genetic dissection of more prevalent essential hypertension. To search systematically for chromosomal regions containing genes that regulate blood pressure, we scanned the entire autosomal genome by using 367 polymorphic markers. Our study population, selected from a blood-pressure screen of >200,000 Chinese adults, comprises rare but highly efficient extreme sib pairs (207 discordant, 258 high concordant, and 99 low concordant) and all but a single parent of these sibs. By virtue of the sampling design, the number of sib pairs, and the availability of genotyped parents, this study represents one of the most powerful of its kind. Although no regions achieved a 5% genomewide significance level, maximum LOD-score values were >2.0 (unadjusted $P < .001$) for regions containing five markers (*D3S2387, D11S2019, D15S657, D16S3396,* and *D17S1303*), in our primary analysis. Other promising regions identified through secondary analyses include loci near *D4S3248, D7S2195, D10S1423, D20S470, D20S482, D21S2052, PAH,* and *AGT.*

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Introduction

The possibility of mapping quantitative-trait loci (QTL) by using sib pairs with extreme phenotypes (hereafter referred to as "extreme sib pairs") has recently generated much attention (Risch and Zhang 1995). This sampling design is widely recognized as being substantially more powerful than a design that uses randomly ascertained sibs (Carey and Williamson 1991; Cardon and Fulker 1994; Risch and Zhang 1995). However, the efficiency of extreme sib pairs is counterbalanced by the large screening effort needed to identify them (Risch and Zhang 1996). Through an international collaboration with researchers in Anqing, China, we have performed such a screen and have completed an initial genome scan in search of genes regulating blood pressure (BP).

In many ways, Anqing is an ideal setting for collection of extreme sib pairs and for the study of BP. First, its population of ~6 million is sufficiently large to contain an adequate number of extreme sib pairs. Second, the combination of a well-established three-tier medical system (county, township, and village) and our extensive research network in China allow us to systematically ascertain these sib pairs. Third, although large, this predominantly rural population is relatively homogeneous. For example, diets and lifestyles tend to be more uniform than those in countries such as the United States. Moreover, genetic diversity has been limited by the rugged terrain and lack of public and private vehicles. Fourth, stable family units allow parental DNA to be collected in many cases. Fifth, BP medication in this part of China is rare, so that the natural variation of BP can more readily be observed.

To attain our extreme sib pairs, we screened >200,000 adults from Anqing. Initially, we identified all study subjects having extreme systolic BP (SBP) or extreme diastolic BP (DBP). To qualify as extreme, a measurement must be in the top or bottom age-specific decile defined on the basis of the first ~40,000 participants (table 1). From this population, we identified extreme discordant sib pairs (EDSPs), high concordant sib pairs (HCSPs), and low concordant sib pairs (LCSPs) and chose those

Table 1

BP Distribution in Anqing Counties of Huaining and Zongyang

Category and Age (No. of Subjects)	BP, at Percentile (mmHg)									Mean BP (SD)
	5%	8%	10%	25%	50%	75%	90%	92%	95%	
SBP:										
15–34 (18,918)	92	96	98	106	114	122	130	132	138	114.8 (13.9)
35–39 (2,246)	92	96	98	106	116	126	136	138	142	117.6 (16.2)
40–44 (4,161)	96	98	100	108	118	128	138	140	148	120.2 (16.9)
45–49 (3,897)	96	98	100	110	120	130	144	148	156	123.1 (18.8)
50–55 (12,154)	100	104	106	118	130	146	166	170	180	134.1 (25.3)
DBP:										
15–34 (18,892)	50	54	56	60	68	76	82	84	86	69.1 (11.2)
35–39 (2,242)	52	56	58	62	70	78	84	86	90	71.3 (11.9)
40–44 (4,159)	54	56	58	64	72	78	88	90	92	72.7 (12.1)
45–49 (3,894)	56	58	60	66	74	80	88	90	94	73.7 (12.4)
50–55 (12,131)	56	58	60	68	76	84	92	96	100	76.1 (14.1)

with available parental DNA (table 2) for inclusion in a genomewide scan for linkage based on 367 autosomal markers and multipoint linkage analysis. In this article, we present the results of our scan and discuss strategies to further investigate several promising regions.

Subjects and Methods

DNA samples and related data were collected in Anqing through the Harvard-Anhui Collaborative Project on Environment, Nutrition, Genetics, and Cardiovascular/Respiratory Diseases, a large-scale epidemiological project initiated in 1993 by scientists from Harvard School of Public Health, Harvard Medical School, Anhui Medical University (AMU), and the Anqing Health Bureau.

Study Site

Spanning 80 km along the north bank of the Yangtze River, Anqing has one major urban area and eight rural counties covering 15,000 km^2. The climate is mild, with average year-round temperatures of ~15°C. Available records indicate settlement 2,000 years ago, and population stability is reflected by strictly regional Anqing dialects. Because divorce and migration are uncommon in Anqing, and because China's single-child policy is not widely enforced, families tend to be large and stable.

BP Distributions and Eligibility Requirements

A community-based survey was conducted on >200,000 residents of Anqing, to collect information on BP, height, and weight. Age-adjusted BP distributions for decile determination were estimated (with ± 1-mm-Hg precision) on the basis of a pilot study comprising the first ~40,000 participants (table 1). These distributions, as well as those from the entire screen (completed in April 1996) were comparable to the distributions from an independent survey conducted in 1993 on >20,000 adults from the same area (Xu et al. 1997). To be eligible for the final phase of our study (i.e., genomic screening), an individual had to meet the following criteria: (1) a resting SBP or DBP (average of three readings) in the top or bottom age-adjusted decile, at each of the three screening stages described below; (2) age 15–55 years; (3) the existence of at least one sib in the same age group who also satisfied criterion (1); and (4) availability of DNA from both parents. Informed consent was obtained from all participants, and the study was approved by the Human Subjects Committee at the Harvard School of Public Health.

Family Ascertainment

A three-stage procedure (done on three separate days) was used to determine which families met the eligibility criteria. First, after receiving rigorous study-specific training and then passing the required proficiency tests, village doctors conducted an initial screening survey. Second, a field team, which included an epidemiologist from the Anhui Meizhong Institute for Biomedical Science and Environmental Health and a group of experienced AMU faculty members, was sent to the villages to confirm BP measurements on families initially found to be eligible. Finally, after positive confirmation, a formal study was conducted at a central office located in the area hospital. Previously validated questionnaires were administered by specially trained interviewers, to gather epidemiological, demographic, and dietary information (Chinese Academy of Preventive Medicine 1990). Anthropomorphic measurements, including height and weight, were taken by trained and certified examiners following standard protocols. BP measurements were obtained after subjects had rested for 10 min. Measurements were made with the patient in the seated position, by nurses using mercury-gravity manometers and appropriately

Table 2

Clinical Characteristics of Study Subjects Used for Genomic Screening

		Offspring	
	Parents	1st Decile, SBP or DBP	10th Decile, SBP or DBP
No.	673	269	508
Male/female	50%/50%	42%/58%	69%/31%
Mean age (SD)	58.9 years (7.6 years)	28.9 (6.1 years)	30.5 (6.6 years)
Proportion taking antihypertensive medication	6%	0%	2%
Mean body-mass index (SD)	21.8 kg/m^2 (6.8 kg/m^2)	20.9 kg/m^2 (2.5 kg/m^2)	22.8 kg/m^2 (3.1 kg/m^2)

sized cuffs. Triplicate measurements on the same arm were taken, with an interval of ⩾30 s between readings. Each patient's SBP and DBP were then calculated as the mean of the three independent measurements. All protocols are fully compatible with standardized methods used in the United States.

Phlebotomy

Fore-arm venous-blood samples were obtained from all study participants via venipuncture. Samples were collected in 10-ml Vacutainer tubes (Becton Dickinson) containing either EDTA (two tubes) or citrate (two tubes). Tubes were kept on ice and subsequently were centrifuged for 10 min in a tabletop refrigerated centrifuge at 4,000 *g*. Plasma was subsequently removed from the cell pellet by pipetting. All samples were frozen and stored at −85°C.

DNA Extraction

DNA extraction was done at the Anhui Meizhong Institute for Biomedical Science and Environmental Health. Isolation of genomic DNA was performed by means of Puragene DNA Isolation Kits (Gentra Systems), by a modification of methods described elsewhere (Buffone and Darlington 1985). This DNA-extraction protocol typically yields 300 μg of DNA from 10 ml of whole blood with an $OD_{260}/OD_{280} \approx 1.8$. After extraction, half of the DNA sample was sent to the Program for Population Genetics, for genetic analysis, and the other half was stored at −85°C, as a backup sample.

Genotyping

Genotyping of Weber screening set 9 markers (Yuan et al. 1997) for 1,450 individuals (777 children from 337 families and 673 of 674 of their parents) was done by the NHLBI Mammalian Genotyping Service in Marshfield, WI (Center for Medical Genetics, Marshfield Medical Research Foundation), by fluorescent-based detection (protocol details can be found at the URL listed in the Electronic-Database Information section). Average heterozygosity was .74, and only three markers had completeness values <90%. For a subset of DNA samples, we repeated the genotyping, in our lab, with seven markers (2,838 total samples) and found 99.8% concordance with the genotypes provided to us.

Analytic Methods

Our analytic strategy can be broadly divided into two classes: primary and secondary analyses. In the primary analysis, we used the ASPEX package, version 1.12, to calculate allele-sharing and maximum LOD score (MLS) values at 3,583 loci (i.e., at all markers and at ~1-cM intervals between them), for EDSPs, HCSPs, and LCSPs. To avoid nonindependence of sib pairs in large sibships, we chose only the first $n - 1$ sib pairs from sibships of size n. Furthermore, we assumed that the probability that sibs share exactly one allele is 50%, thereby reducing the number of free parameters to one (i.e., the probability that sibs share either zero or, equivalently, two alleles). We verified that this assumption is reasonable for a wide range of scenarios, by deriving the expected allele sharing for an extensive collection of quantitative genetic models. We found that, in the overwhelming majority of cases, sharing of exactly one allele was close to 50% (authors' unpublished data). Moreover, it has been shown, for qualitative traits (Lunetta and Rogus 1998), that the simplifying assumption that 50% share exactly one allele results in a generally valid test that is typically more powerful than a triangle-type test (Holmans 1993), even if this assumption is not strictly satisfied.

Genomewide significance levels were calculated for the primary analysis by using a resampling technique developed by Rogus et al. (in press). In this procedure, the likelihood is approximated by an asymptotically equivalent expression that can be easily resampled via successive simulation of independent normal realizations. In brief, the LOD score assuming that 50% of cases share exactly one allele can be expressed as $W = \frac{1}{2}[(\Sigma\tau_j)^2/(\Sigma\tau_j^2)]$, where $\tau_j = (Z_{2j} - Z_{0j})/[(Z_{2j} - Z_{0j})Z_2 + \frac{1}{2}(Z_{0j} + \frac{1}{2}Z_{1j})]$, Z_{ij} is the probability that the ith pair shares j alleles (conditional on the data) and Z_2 is the overall probability that sib pairs (e.g., EDSPs) share two alleles. Under the null hypothesis, this is asymptotically equivalent to $W^* = \frac{1}{2}[(\Sigma\tau_j G_j)^2/(\Sigma\tau_j^2)]$, where the G_j are

Table 3

Promising Regions Identified through Primary Analysis

Phenotype and Sib-Pair Type	Marker Nearest Peak Multipoint MLS	Multipoint MLS (Single-Point MLS)	Multipoint *P* (Adjusted)
SBP:			
EDSP	D11S2019 (GGAA5C04)	2.07 (2.56)	.0010 (>.20)
HCSP	D17S1303 (GATA64B04)	2.16 (2.35)	.0008 (>.20)
LCSP	D3S2387 (GATA22G12) (4 cM telomeric to peak)	2.03 (1.61)	.0011 (>.20)
LCSP	D16S3396 (ATA55A11)	2.74 (1.68)	.0002 (.07)
DBP:			
LCSP	D15S657 (GATA22F01) (9 cM centromeric to peak)	2.69 (2.26)	.0002 (.08)

independent and identically distributed from the standard normal distribution. To find a genomewide critical value c_α across s loci such that $P(\sup_s W(s) \geq c_\alpha \mid \text{no linkage}) = \alpha$, it is possible to simulate $W^*(s)$ repeatedly (merely by simulating new sets of G_j) and to determine c_α so that the proportion of replicates with a $W^*(s)$ exceeding c_α is α.

Secondary analyses were also performed—first, to validate the results by means of a second software package, a modified version of MAPMARKER/SIBS, version 4 (Kruglyak and Lander 1995*a*), and, second, to search for additional hints of linkage, by varying the statistics used and the assumptions made. For example, as an alternative to considering only the first $n - 1$ independent pairs, we also considered all available pairs, using MAPMAKER/SIBS (modified) to compensate for nonindependence, by weighting each pair by the factor $2/n$ (Suarez and Hodge 1979). (Our extensively tested modifications to MAPMAKER/SIBS were made to accommodate EDSPs, whose sharing will be less than usual, given linkage. Because we assume that 50% of cases share exactly one allele, this programming change simply requires allowing that the sharing of zero alleles be $\geq$25%, as opposed to $\leq$25%). We also conducted secondary and diagnostic analyses by using mean tests (Blackwelder and Elston 1985), unconstrained 2-df tests (Olson 1997), and single-point (as opposed to multipoint) tests. Although we did not expect 367 markers to offer sufficient coverage of the genome to allow for linkage disequilibrium to be detected with high probability, we also routinely calculated transmission/disequilibrium test (TDT) statistics, because their determination in ASPEX is trivial. In the TDT analysis, we simply searched, via McNemar's statistic, for frequent (or infrequent) transmission of alleles to either top-decile or bottom-decile offspring. For each of our 367 markers, we identified each allele heterozygous in at least five parents and compared transmission versus nontransmission from the heterozygous parents (see Schaid 1996). The total number of alleles considered—and, hence, the number of tests performed—was 2,464–3,122, depending on the type of sib pairs under consideration. Bonferroni adjustments were made on the basis of these numbers. Because we used the TDT to test for linkage—and not to test for association in the presence of linkage (i.e., fine mapping)—no correction was needed for multiple offspring within a family (Spielman et al. 1993). For multipoint linkage analysis, we took genetic distances from the sex-averaged genetic maps provided by the Center for Medical Genetics, Marshfield Medical Research Foundation, and we assessed observed versus expected recombinations, as well as information content, by using GENEHUNTER (Kruglyak et al. 1996).

Results

Primary Analysis

Although no chromosomal region was able to achieve 5% genomewide significance, analyses based on either SBP alone (70 EDSPs, 39 LCSPs, and 185 HCSPs—70, 32, and 159 of them independent, respectively) or DBP alone (95 EDSPs, 67 LCSPs, and 83 HCSPs—94, 54, and 73 of them independent, respectively) revealed several promising regions (table 3 and fig. 1). For SBP, we found MLS values of 2.07 at *D11S2019* (in EDSPs), 2.16 at *D17S1303* (in HCSPs), 2.03 located 4 cM from *D3S2387* (in LCSPs), and 2.74 at *D16S3396* (in LCSPs). For DBP, an MLS of 2.69 was achieved 9 cM from *D15S657* (in LCSPs). However, when we included all offspring with extreme BP (i.e., all offspring having either extreme SBP, extreme DBP, or both), no notable MLS values were observed. Specifically, we tested 203 independent EDSPs, 80 independent LCSPs, and 215 independent HCSPs, at 3,583 locations across the genome, but no locus yielded MLS > 2.0. MLS values were based on statistics for the sharing of exactly two alleles (Risch 1990), as implemented by the ASPEX package.

Although we focused on regions with a primary analysis where MLS > 2.0, this was somewhat arbitrary. We therefore investigated genomewide significance levels for the specific multiple correlated tests in our analysis. To avoid assumptions implicit in general guidelines (Lander

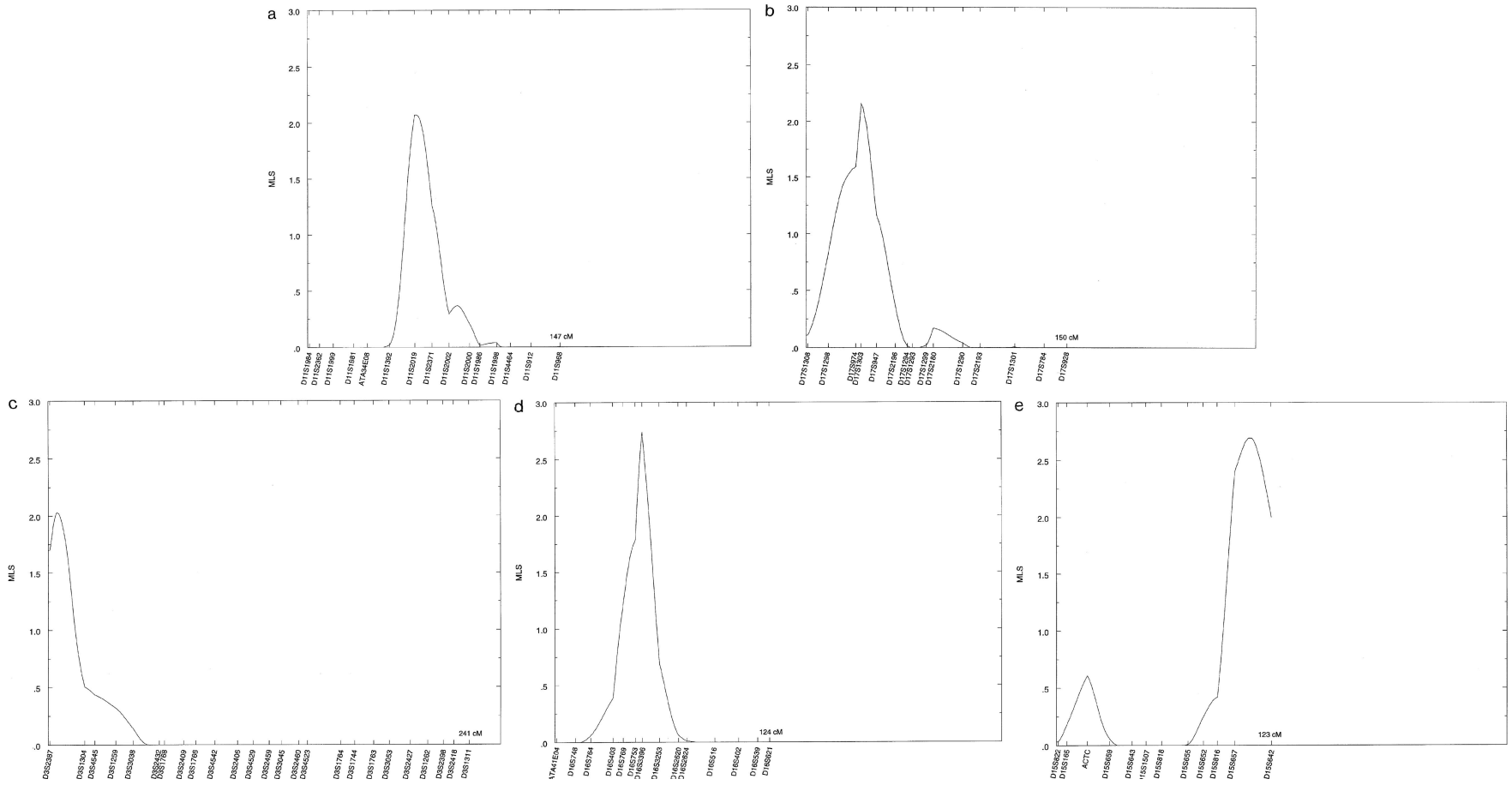

Figure 1 Chromosomes with MLS > 2.0 in the primary analysis. *a*, Chromosome 11 for SBP in EDSPs. *b*, Chromosome 17 for SBP in HCSPs.*c*, Chromosome 3 for SBP in LCSPs. *d*, Chromosome 16 for SBP in LCSPs. *e*, Chromosome 15 for DBP in LCSPs.

and Kruglyak 1995), we applied a resampling procedure (Rogus et al., in press) to our data and found that, for all our scenarios, MLS ≈ 2.9 corresponds to a 5% genomewide false-positive rate. Our largest MLS, 2.74 on chromosome 16 (SBP in LCSPs) corresponds to a genomewide *P* value of .07. Other adjusted *P* values can be found in table 3.

Secondary Analysis

Secondary analyses were performed with an alternate computer program, MAPMAKER/SIBS (Kruglyak and Lander 1995*a*) and with other statistics and assumptions. Although the change in computer program did not affect results (99.9% of MLS values matched at the level of <0.1 unit), some of the statistical modifications did. For example, the use of mean-sharing statistics (Blackwelder and Elston 1985) produced a notably higher MLS, 3.22, for the chromosome 15 region reported above. Otherwise, except for some slightly lower peaks, the mean-sharing statistics produced MLS profiles quite similar to those produced by the primary analysis. We also repeated analyses by using all available (vs. independent) sib pairs, with appropriate weights (Kruglyak and Lander 1995*a*). Similar MLS profiles resulted, although peak scores were slightly lower, and three additional regions surfaced: MLS = 1.98 near *D20S470* (either SBP or DBP in HCSPs), MLS = 2.33 near *D10S1423* (DBP in HCSPs), and MLS = 2.20 near *D21S2052* (DBP in LCSPs).

To ensure that our multipoint analysis was not unduly impacted by genotyping error–induced recombinations, we next performed single-point analysis. Peak MLS locations corresponded well (i.e., the difference was <1 marker) with those from multipoint analysis. Two single-point MLS values did exceed their multipoint counterparts (see table 3), and a novel region (MLS = 2.38) at *D4S3248* emerged for SBP in HCSPs.

In addition to the 1-df (two-allele and mean-sharing) tests described above, we also performed 2-df tests (Olson 1997) on our data. Albeit larger, owing to an extra 1 df, the 2-df MLS profiles implicate essentially the same regions: two were somewhat less significant (unadjusted *P*. ≈ 006 for both), and two additional regions had 2-df MLS > 3.0, roughly equivalent to 1-df MLS > 2.0: MLS = 3.01 (either SBP or DBP in EDSPs) 2 cM from *D7S2195* (unadjusted *P* = .000977), and MLS = 4.96 (SBP in HCSPs) 5 cM from *D20S482* (unadjusted *P* = .000011). (We present the unadjusted *P* values for these secondary analyses as a way to compare the 2-df results with the primary, 1-df results.) The latter region, however, is peculiar because of an excess of sibs sharing one allele (Olson 1997).

Finally, we calculated a simple TDT statistic for each marker allele in the screen. Although peak TDT scores obviously reflect the multitude of statistics considered, we note that the third highest (TDT = 17.9; Bonferroni adjusted exact *P* = .086; top-decile SBP/DBP offspring) occurs at *D16S3253*, one marker from our best primary-analysis MLS. A weak signal (unadjusted exact *P* = .002 [nonsignificant adjusted *P* value]) was also detected at *D7S2195* (bottom-decile SBP), the same locus identified via 2-df analysis.

Potential Candidate Genes

Recognizing that peak MLS values may not occur at the precise locus involved in a polygenic disease (Kruglyak and Lander 1995*b*), we identified several candidate genes in the vicinity of our most promising scores. *NOS3* (for nitric oxide synthase 3) and *ABP1* (for amiloride-binding protein-1) map close to the highest MLS on chromosome 7. *AGTRL1* (for angiotensin receptor-like 1) and *HUMPCP* (*PRCP*) (for human angiotensinase C [prolylcarboxypeptidase]) are within reasonable distances of *D11S2019*. Although chromosome 16 contains many potentially relevant genes, only *SLC12A3* (for thiazide-sensitive Na-Cl cotransporter) is close to *D16S3396*.. Both the region homologous to the recently reported rat susceptibility region (Julier et al. 1997) and *PHA2B* (for pseudohypoaldosteronism type II) are the closest of the candidate genes to *D17S1303*. *ADRA1D* (for alpha-1D-adrenergic receptor) and *AVP* (for arginine vasopressin) could be candidate genes in proximity to the highest MLS on chromosome 20. Other candidates include *CHRM5* and *CHRNA3-5* (for cholinergic receptors) and *TFCOUP2* (for apolipoprotein-regulatory protein) on chromosome 15 and *ATP2B2* (for ATPase Ca^{2+}-transporting plasma membrane) on chromosome 3.

One frequently studied candidate gene for BP is that for angiotensinogen (AGT). Interestingly, when we selected subjects with both high DBP (Jeunemaitre et al. 1992) and hypertensive parents (Watt et al. 1992), analysis of HCSPs (25 independent sib pairs) revealed a sharp peak (MLS = 2.64) in close proximity to *AGT*. The same sample, admittedly small but potentially genetically enriched, also provides results pointing to a region of chromosome 12 (MLS = 2.76), a possible hypertension-susceptibility QTL (Frossard and Lestringant 1995) in the vicinity of the *PLA2G1B* gene.

Discussion

BP regulation is a complex process influenced by numerous genetic and environmental factors. Consequently, there is no cookie-cutter approach for elucidation of the genetic mechanisms involved. We attacked this problem by scanning the genome for linkage by using extreme sib pairs. We chose a genome scan because

previous candidate-gene studies in humans have left much unexplained about the variation in BP. We chose linkage analysis because, at the present time, scanning the genome for association is not feasible. Finally, we chose to perform our scan on extreme sib pairs in order to extract maximal information from our genotyping effort. Our three sib-pair types were analyzed separately for two reasons. First, because different types of sib pairs are better for different genetic models, the combining of sib pairs can result in a masking of an otherwise strong linkage result. Second, unless reliable knowledge of the genetics model is available, it is unclear how best to combine sib pairs. We are, however, in the process of conducting quantitative analysis that will combine all data in a single analysis.

Our extreme sib pairs were chosen from a relatively homogeneous area of China, where BP medication is uncommon. Furthermore, in all but a single case, parental DNA was available, thus improving information content for linkage analysis (Kruglyak and Lander 1995*a*) and allowing for TDT analysis.

Five promising regions were identified via our primary analysis, and eight others were identified via secondary analyses. Because none of the scores reached 5% genomewide significance, we are now in the process of genotyping additional extreme sib pairs, as part of an extension study. In general, these additional families lack DNA from either one or both parents. However, the density of the markers in the extension study will compensate for these missing data.

Because our original intuition suggested that we might see coincident linkages, we did not apply any additional correction for either multiple sib types or multiple phenotype definitions. As discussed above, quantitative analysis, which utilizes all data simultaneously, could eliminate this issue to some extent, although it is unclear whether this is a powerful strategy. If, for example, different genes are involved in SBP versus DBP or in high BP versus low BP, then pooling of the data may be suboptimal. (Also, adjustment for either multiple sib types or multiple phenotype definitions in this case is not indicated. Thus, we present results for each genome scan separately, along with the caveat that multiple scans have been performed.)

In this study, extreme phenotypes were defined as those within the top and bottom age-adjusted population deciles. We chose to use deciles not only because Risch and Zhang (1995) discussed this approach at great length but also because we did not foresee an excess of high-BP (compared with low-BP) subjects, because our study was population based. Although the cost and complexity of the data collection prevented us from adjusting our strategy midstream, it would, perhaps, have been beneficial to relax the lower extreme cutoff, as suggested by Risch and Zhang (1996). In the extension study, we are investigating a more refined definition of extreme, which takes into account additional factors such as sex and body weight. Moreover, although some previous studies, particularly those focused on hypertension, have considered a joint phenotype based on SBP *or* DBP, our extension study will treat these two measurements separately. In this way, we will concentrate on those phenotypes that have yielded the best linkage scores and, simultaneously, will allow for different underlying biological mechanisms.

In our study, the smallest *P* value adjusted for multiple comparisons across any given genome was 0.07. However, we should point out that we also considered three types of sib pairs and two BP measurements (although these scans are clearly not independent). Thus, the adjusted *P* values are even less striking, and the lack of highly significant MLS values in our study demonstrates just how difficult an undertaking genomewide searches for genes influencing complex traits can be. Indeed, even though (*a*) EDSPs have been shown to be tremendously powerful, via theoretical work (Risch and Zhang 1995) and simulation (Rogus et al. 1997), (*b*) our population was homogeneous, and (*c*) information was collected on all parents, we were unable to unequivocally locate genomic regions in this study. Future studies will need to address this, by collecting larger samples of selected sib pairs, reducing further any environmental and genetic heterogeneity, and defining more carefully either the phenotype of BP or, perhaps, relevant intermediate phenotypes. In addition, association studies can be considered for candidate regions identified on the basis of either biological function or linkage analysis.

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Electronic-Database Information

URLs for data in this article are as follows:

ASPEX, ftp://lahmed.stanford.edu/pub/aspex/index.html
Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics

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