ARTICLE

A Chromosome 11q Quantitative-Trait Locus Influences Change of Blood-Pressure Measurements over Time in Mexican Americans of the San Antonio Family Heart Study

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Although previous genome scans have searched for quantitative-trait loci (QTLs) influencing variation in blood pressure (BP), few have investigated the rate of change in BP over time as a phenotype. Here, we compare results from genomewide scans to localize QTLs for systolic, diastolic, and mean arterial BPs (SBP, DBP, and MBP, respectively) and for rates of change in systolic, diastolic, and mean arterial BPs (rSBP, rDBP, and rMBP, respectively), with use of the longitudinal data collected about Mexican Americans of the San Antonio Family Heart Study (SAFHS). Significant evidence of linkage was found for rSBP (LOD 4.15) and for rMBP (LOD 3.94) near marker *D11S4464* located on chromosome 11q24.1. This same chromosome 11q region also shows suggestive linkage to SBP (LOD 2.23) and MBP (LOD 2.37) measurements collected during the second clinic visit. Suggestive evidence of linkage to chromosome 5 was also found for rMBP, to chromosome 16 for rSBP, and to chromosomes 1, 5, 6, 7, and 21 for the single-time-point BP traits collected at the first two SAFHS clinic visits. We also present results from fine mapping the chromosome 11 QTL with use of SNP-association analysis within candidate genes identified from a bioinformatic search of the region and from whole-genome transcriptional expression data collected from 1,240 SAFHS participants. Our results show that the use of longitudinal BP data to calculate the rate of change in BP over time provides more information than do the single-time measurements, since they reveal physiological trends in the subjects that a single-time measurement could never capture. Further investigation of this region is necessary for the identification of the genetic variation responsible for QTLs influencing the rate of change in BP.

Hypertension is a leading cause of death and morbidity in our society,[1] because it leads to stroke, heart and renal failure, and death due to cardiovascular disease (CVD).[2–7] Blood-pressure (BP) variation is influenced by both genetic and environmental factors.[8,9] Previous studies show that BP increases with age at different rates.[10,11] The rise in systolic (SBP) and diastolic (DBP) BPs is likely due to increased peripheral resistance.[12] Mean arterial BP (MBP) is highly correlated with SBP and DBP and often describes the overall variation in BP.[13] Data from studies suggest that MBP is a stronger indicator of CVD risk than is pulse pressure[14] or SBP,[13] particularly when individual BP parameters in adults aged <60 years are considered.

Longitudinal studies are efficient designs for the investigation of individual changes, such as health and BP status, over time.[15] Longitudinal twin and family studies demonstrate a substantial genetic contribution to the change in BP over time.[16,17] The concept of "variable genes," whose expression depends on environmental exposure, is proposed to explain the longitudinal trends in BP within each person.[18,19] Genetic and epidemiological BP studies show that use of serial measurements of BP in study participants at multiple time points reduces measurement error and minimizes short-term effects, because single BP measurements are subject to variation from time to time within the same individual.[17,19–23] In addition, detection of linkage may be enhanced by using longitudinal BP phenotypes as a quantitative trait, because genes that affect BP can contribute not only to elevated BP but also to intermediate and low BPs.[21] Therefore, the identification of BP-susceptibility loci may be enhanced by analyzing measurements of both long-term levels and trends in BP. Furthermore, it has been proposed that there is a need to investigate genetic loci for both levels and rate of change in BP over time.[17]

Although many genomewide scans have been conducted on BP phenotypes, few have investigated rate of change in BP traits, possibly because of limited availability of lon-

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Table 1. Characteristics of the Study Sample

Sex and Characteristic	Visit 1[a]	Visit 2[b]	Yearly Change[b]
Men:			
No. of subjects	284	255	255
Age, in years	35.5 (.92)	39.0 (.93)	5.0 (.09)
SBP	121.2 (.86)	123.4 (.94)	.7 (.19)[c]
DBP	75.4 (.57)	72.6 (.64)	−.1 (.17)
MBP	88.6 (.58)	89.5 (.67)	.2 (.16)
BMI[d]	28.6 (.35)	29.5 (.38)	.2 (.04)[c]
Waist circumference, in mm	963.0 (10.25)	998.2 (9.30)	10.0 (1.14)[c]
Triglycerides	1.76 (.08)	1.63 (.09)	.00 (.02)
Total cholesterol	4.86 (.06)	4.62 (.06)	−.04 (.01)[c]
HDL cholesterol	1.26 (.02)	1.22 (.02)	−.01 (.00)
Women:			
No. of subjects	455	406	406
Age, in years	36.5 (.68)	39.8 (.70)	5.2 (.07)
SBP	115.4 (.80)	118.9 (.85)	1.1 (.12)[c]
DBP	68.8 (.46)	70.1 (.50)	.3 (.11)
MBP	84.3 (.51)	86.3 (.56)	.6 (.10)[c]
BMI[d]	29.7 (.34)	30.5 (.36)	.2 (.04)[c]
Waist circumference, in mm	919.1 (8.30)	975.3 (8.54)	14.0 (1.26)[c]
Triglycerides	1.49 (.05)	1.46 (.06)	.01 (.01)
Total cholesterol	4.83 (.04)	4.53 (.04)	−.05 (.01)
HDL cholesterol	1.36 (.02)	1.28 (.02)	−.02 (.00)

NOTE.—All values are mean (SE). BP is measured in mm Hg. Cholesterol is measured in mmol/liter.

[a] Excludes individuals who reported treatment with antihypertensive medication at visit 1; lipid estimates also exclude individuals ($n = 7$) who reported treatment with antihyperlipidemia medication at visit 1.

[b] Excludes individuals who reported treatment with antihypertensive medication at either visit; lipid estimates also exclude individuals ($n = 18$) who reported treatment with antihyperlipidemia medication at either visit.

[c] Significantly different from 0 at $P < .003125$ (one-tailed t test, $\alpha = .05$ with Bonferroni correction for eight tests per sex).

[d] Calculated as weight in kilograms divided by the square of height in meters.

gitudinal data and limitations in analytical methods.[24] Even fewer studies have investigated MBP over time as a trait for genomewide scans. We decided to compare results from a genomewide scan that used the rates of change in SBP (rSBP), DBP (rDBP), and MBP (rMBP) (calculated as the difference between two measures in two clinic visits divided by the time interval between visits) with results from a genomewide scan that used the single-time-point BP measurements collected from Mexican American participants of the San Antonio Family Heart Study (SAFHS). In addition, we show results from a novel exploratory fine-mapping approach that makes use of a combination of gene-expression data, bioinformatics, and SNP-association analyses in this Mexican American population.

Subjects and Methods

SAFHS Population

The subjects in this study were Mexican American participants of the SAFHS.[25] Probands and their relatives were invited to the clinic once during 1990–1992 (first clinic visit), again during 1994–1996 (second clinic visit), and for a third time during 2004–2006. Over 1,400 subjects from 42 large, extended families were recruited in clinic visit 1. Families were recruited, without regard to disease status, around an index case (proband) who was Mexican American and aged 40–60 years. To assure large families, probands were required to have a spouse and at least six age-eligible offspring and/or siblings living in San Antonio. Information about medical history, family relationship, lifestyle, and anthropometry were obtained, and blood samples were drawn from the participants. BPs were measured three times on the left arm by a random-zero sphygmomanometer, and the averages of the latter two measures were used as trait values. MBP was calculated using the formula MBP = 1/3(SBP) + 2/3(DBP).

Of the 817 subjects with BP data collected on the first and second clinic visits, we used data from 739 of these subjects for the first-clinic-visit BP genome scan after excluding individuals who reported treatment with antihypertensive medication at that visit. We used 661 of these subjects for the longitudinal and the second-clinic-visit BP genomewide scans after excluding individuals who reported treatment with antihypertensive medication at either clinic visit.

Body weight, height, and waist and hip circumferences were recorded according to standard protocol,[26] and lipid panels were measured with a Ciba-Corning Express Plus clinical chemistry analyzer with commercial reagents (Roche Diagnostics and Stanbio). The high-density lipoprotein (HDL) cholesterol was quantified in plasma samples after precipitation of β lipoproteins with dextran sulfate.[27] All protocols in this study were approved by the Institutional Review Board of the University of Texas Health

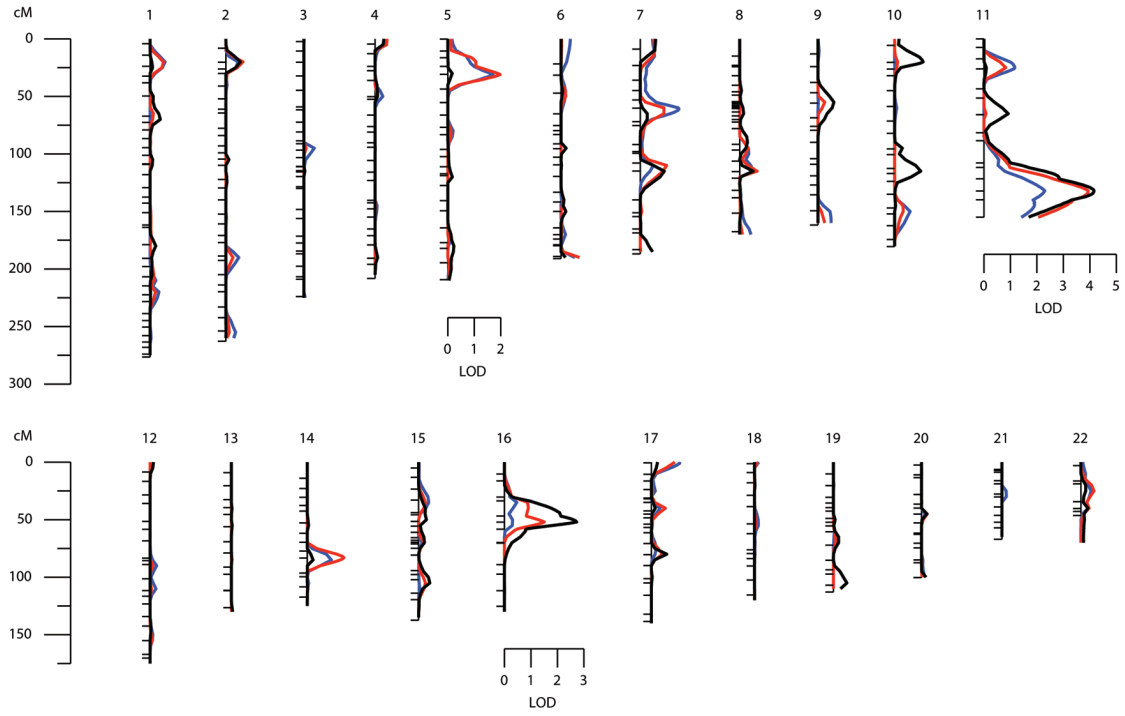

Figure 1. Multipoint linkage analysis of rate of change in BP traits, with use of a 10-cM genome scan of Mexican Americans of the SAFHS. Traits analyzed are rSBP (*black*), rDBP (*blue*), and rMBP (*red*). The horizontal scale shows LOD scores >1.5, and the vertical scale shows distance (cM).

Science Center at San Antonio, and all participants gave informed written consent.

Genomewide Screening

Lymphocytes were isolated from whole blood, and DNA was extracted for genotyping. For the genomewide scan, genomic DNA was amplified by PCR with use of 432 fluorescently labeled primers from MapPairs 6 and 8 Linkage Screening Sets (Research Genetics). The PCR products were pooled and analyzed using an automated DNA sequencer (Applied Biosystems [ABI] model 377, with Genescan and Genotyper programs).

Multipoint linkage analyses were conducted using the variance-decomposition method implemented in SOLAR (Hlod).[28,29] The variance-components linkage method is based on classic quantitative genetic principles, in which the phenotype is assumed to be influenced by both genetic and environmental factors. The genetic factors can include effects of shared alleles at specific loci at defined chromosomal locations. Evidence of genetic effects is obtained from the observed covariances among different classes of relatives.

The observed phenotypic covariances among individuals are decomposed into partial covariances on the basis of allele sharing at specific loci (on the basis of marker genotypes), allele sharing elsewhere in the genome (on the basis of kinship), and nonadditive genetic factors. If the variance component for a specific chromosomal location is significantly greater than zero, there is evidence of a locus influencing the phenotype at that location. In practice, we tested for the existence of a locus influencing the phenotype at intervals across the entire autosomal genome.

Parameter estimation and hypothesis testing are performed using a maximum-likelihood framework, under the assumption of multivariate normality. For each BP trait, we test the null hypothesis that the additive genetic variance due to a specific QTL equals zero (no linkage) by comparing the likelihood of this restricted model with that of a model in which the variance due to this QTL is estimated.

The difference between the two $\log_{10}$ likelihoods yields a LOD score, which measures the support for the hypothesis of linkage over that of "no linkage" at a given chromosomal location. A LOD score of 3.0, for example, indicates that the statistical support for the linkage hypothesis is 10^3 times that for the null hypothesis. Since this represents a pointwise $P < .05$ corrected for the multiple tests in a typical genome scan, LOD ≥ 3 is customarily taken as significant evidence of linkage.[30] P values for this test are obtained from twice the difference in $\log_e$ likelihoods of these two models, which yields a test statistic that is asymptotically distributed as a (1/2):(1/2) mixture of a χ^2 variable and a point mass at zero.[31]

Data for the longitudinal BP study were transformed either by natural-logarithm transformation or inverse-normalization procedures (rSBP, rDBP, and rMBP), to reduce skewness and kurtosis (leptokurtic distributions may increase the type 1 error rate in multipoint analyses).[32] The inverse normal transformation of a data set is performed by the following procedure. First, the trait values are sorted, and, for any value V found at position I in the sorted list, a quantile is computed for it by the formula $I/(N+1)$. The inverse-normal-cumulative-density function is computed for each quantile and is stored in an array keyed by ID and by family ID, if applicable. When the value V occurs multiple times, the inverse normal is computed for each applicable quantile and

Table 2. Genome-Scan Results with LOD Scores ⩾1.9

Trait[a] and Chromosome	Location[b] (cM)	Nearest Marker	LOD
rMBP:			
5	31	*D5S817*	2.0
11	132	*D11S4464*	3.94
rSBP:			
11	132	*D11S4464*	4.15
16	52	*D16S769*	2.73
rDBP:			
11	133	*D11S4464*	2.27
MBP2:			
1	203	*D1S1660–D1S1668*	2.34
5	192	*D5S1456–D5S211*	1.94
11	105	*D11S2000*	2.37
11	117	*D11S1998*	2.17
SBP2:			
1	193	*D1S1660–D1S1668*	2.08
5	187	*D5S1456–D5S211*	2.53
7	165	*D7S1805*	2.06
11	117	*D11S1998*	2.23
DBP2:			
5	192	*D5S1456–D5S211*	1.97
11	101	*D11S2000*	2.63
MBP1:			
21	21	*D21S1437*	2.44
SBP1:			
6	83	*D6S1053*	2.15

[a] Traits analyzed: rSBP, rDBP, and rMBP; SBP (SBP2), DBP (DBP2), and MBP (MBP2) from clinic visit 2; and SBP (SBP1), DBP (DBP1), and MBP (MBP1) from clinic visit 1.

[b] Locations are based on the deCODE map.

is averaged, and then the average is stored for each ID. We incorporated sex, age at visit 1, and the inverse-normal-transformed rate of change in BMI (rBMI) as covariates into the longitudinal analysis.

For the single-time-point BP-measurement genome scan, we used inverse-normal-transformed data for SBP, DBP, and MBP at each clinic visit and incorporated sex, age at that visit (1 or 2), and inverse-normal-transformed BMI at that visit (1 or 2) as covariates in the analysis.

We also tested for locus heterogeneity when significant evidence of linkage was obtained for a particular locus. We used the heterogeneity LOD (HLOD) routine in SOLAR to perform a classic heterogeneity test under a model allowing for two sets of families, with only one set segregating the locus of interest. The distribution of the test statistic for homogeneity conditional on linkage is taken to be a 50:50 mixture of a χ^2 distribution with 1 df and a point mass at zero (HLOD).

Gene-Expression Analysis

RNA was extracted from lymphocytes isolated from whole blood available from 1,280 SAFHS participants. The gene-expression data presented here were acquired as part of the whole-genome gene-expression analysis in the SAFHS (H.H.H.G., J.E.C., M.P.J., T.D.D., S.A.C., J.B.J., D. L. Rainwater, A.G.C., M. C. Mahaney, L. Almasy, J.W.M., G. R. Collier, E.K.M., and J.B., unpublished data). In summary, expression levels of 47,289 different transcripts from 1,280 members of 42 SAFHS Mexican American families were tested using Illumina's Sentrix Human-6 gene-expression bead chips (v. 1). We identified 20,413 transcripts that exhibited significant expression in 1,240 SAFHS subjects. Genomewide variance components–based linkage analysis was performed on the expression levels of all transcripts (H.H.H.G., J.E.C., M.P.J., T.D.D., S.A.C., J.B.J., D. L. Rainwater, A.G.C., M. C. Mahaney, L. Almasy, J.W.M., G. R. Collier, E.K.M., and J.B., unpublished data). Heritability estimates were also obtained for each transcript (H.H.H.G., J.E.C., M.P.J., T.D.D., S.A.C., J.B.J., D. L. Rainwater, A.G.C., M. C. Mahaney, L. Almasy, J.W.M., G. R. Collier, E.K.M., and J.B., unpublished data). For the present study, regression analyses were performed to estimate the mean effect of transcripts from genes within the chromosome 11 linkage region. For each BP phenotype, each transcript was tested separately as a covariate in the peak linkage model, to estimate both the significance of the regression (as probability that the regression slope = 0) and the change in the QTL-specific variance (conditional linkage).[33]

Bioinformatic Analysis

We used the computer software GeneSniffer to investigate the potential relevance of each gene in our region to BP.[34] GeneSniffer identifies all known and predicted genes within a defined chromosome region by mining bioinformatics databases from the National Center for Biotechnology Information Entrez Gene, OMIM, and PubMed, to objectively identify plausible positional candidate genes. Interrogation of these databases is performed using a set of disease-specific keywords that are assigned a score (scale 1–10, with 10 as the greatest) on the basis of their relevance and significance to a particular phenotype of interest.[34] GeneSniffer also performs analysis to identify genes homologous to those in the region of interest. Homologues of each gene within the region of interest are identified by BLAST and are scored for content in their Entrez Gene, OMIM, and PubMed entries. Each score is weighted in accordance with the degree of homology, and a cumulative "hit score" is calculated for each gene. Scores generated for both the relevance to keywords and to homology of other genes are documented in an HTML format for all genes

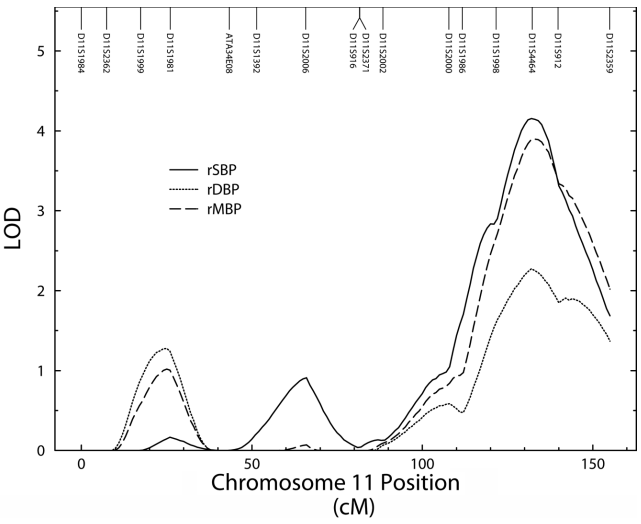

Figure 2. Multipoint linkage analysis of rate of change in BP traits, with use of a 10-cM genome scan of chromosome 11 in Mexican Americans of the SAFHS. Tick marks indicate marker locations.

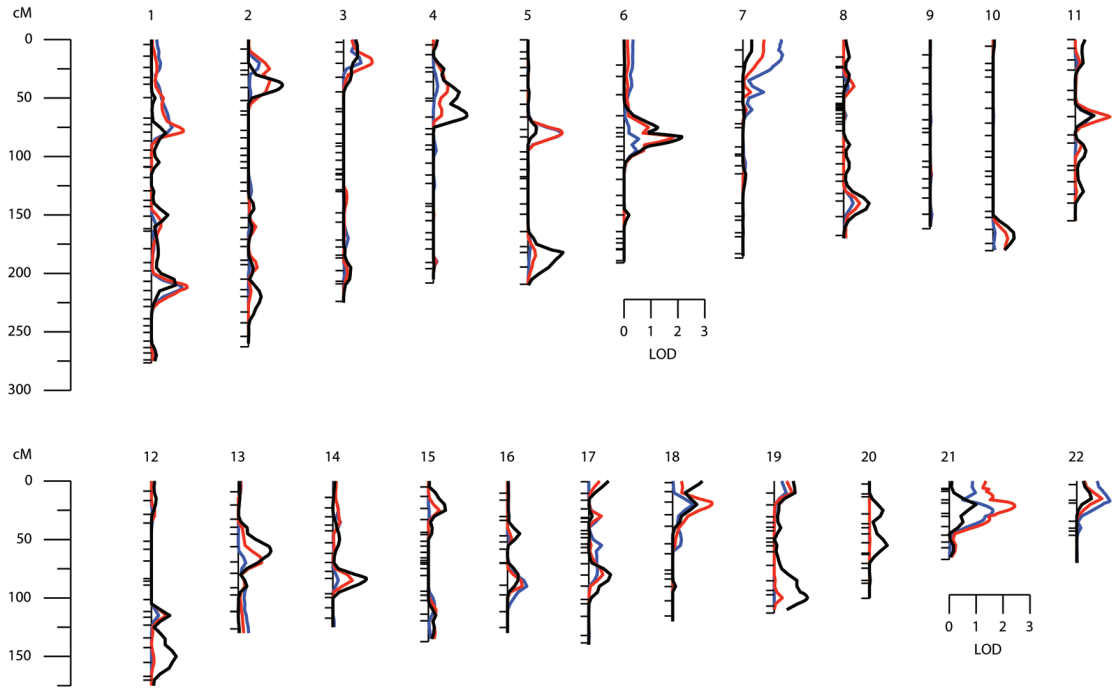

Figure 3. Multipoint linkage analysis of BP traits from clinic visit 1, with use of a 10-cM genome scan of Mexican Americans of the SAFHS. Traits analyzed are SBP (*black*), DBP (*blue*), and MBP (*red*) collected at clinic visit 1. The horizontal scale shows LOD scores >1.5, and the vertical scale shows distance (cM).

within the region of interest. Also listed in the HTML format are the source of database hits and links to external databases for additional information. GeneSniffer therefore provides a quantitative indicator of the relevance of a gene to a particular phenotype of interest.

SNP Analysis

SNPs located in coding and regulatory regions of genes were preferentially selected for genotyping and analysis. Special priority was given to SNPs that predict functional changes in these genes—for example, missense, nonsense, and splice-site mutations. SNPs that were experimentally validated by HapMap or by Applera Genome Resequencing Initiative were chosen for further evaluation. For this reason, primers and probes for TaqMan allelic discrimination assays of 85 validated SNPs were purchased either from the ABI Predesigned or Validated and Coding SNP Genotyping Assays. TaqMan reactions contained 10 ng genomic DNA in a 5-μl reaction volume containing 1x TaqMan Universal PCR Master Mix without AmpErase uracil-N-glycosylase (2x), the PCR primers, and both the FAM and VIC reporter-labeled TaqMan MGB probes for the SNP at 1x final concentration. The amplification conditions consisted of an initial incubation step at 95°C for 10 min, followed by 40 cycles at 92°C for 15 s and then at 60°C for 1 min, with use of an ABI Prism 7900 sequence-detection system.

An allelic-transmission scoring method (the QTL disequilibrium test [QTLD]) incorporated into SOLAR Hlod[35] was used to test the 85 SNPs for association with each BP phenotype. We tested for population stratification for each SNP by comparing the likelihood of a model in which the association parameters are estimated separately for each pedigree with one in which they are constrained to be equal, as would be expected in the absence of population stratification. In the presence of population stratification, we conducted a quantitative transmission/disequilibrium test (QTDT)[36] incorporated into QTLD for this contingency.[35] Age and sex were incorporated into all analyses as covariates.

Results

Genomewide Scan of Single-Time-Point and Longitudinal BP Traits

Table 1 shows the characteristics of the male and female subjects used in this study at both clinic visits. Since the studied population was getting older during the 3–4 years between visits 1 and 2, positive average yearly changes of BMI, waist circumference, triglycerides, SBP, and MBP were observed in both males and females. Negative average yearly changes were seen in measurements of HDL and total cholesterol levels in both sexes. Changes in BMI, waist circumference, SBP, and MBP in females and in BMI, waist circumference, total cholesterol, and SBP in males were significantly different from zero (table 1). We observed minor differences in DBP in both sexes, but these changes were not significant (table 1). It is important to note that the mean differences in table 1 are not adjusted for age or BMI, as are the phenotypes in the genetic analyses, and do not take into account the kinship between the subjects. Thus, table 1, although not a formal epidemiological comparison (because the subjects are related

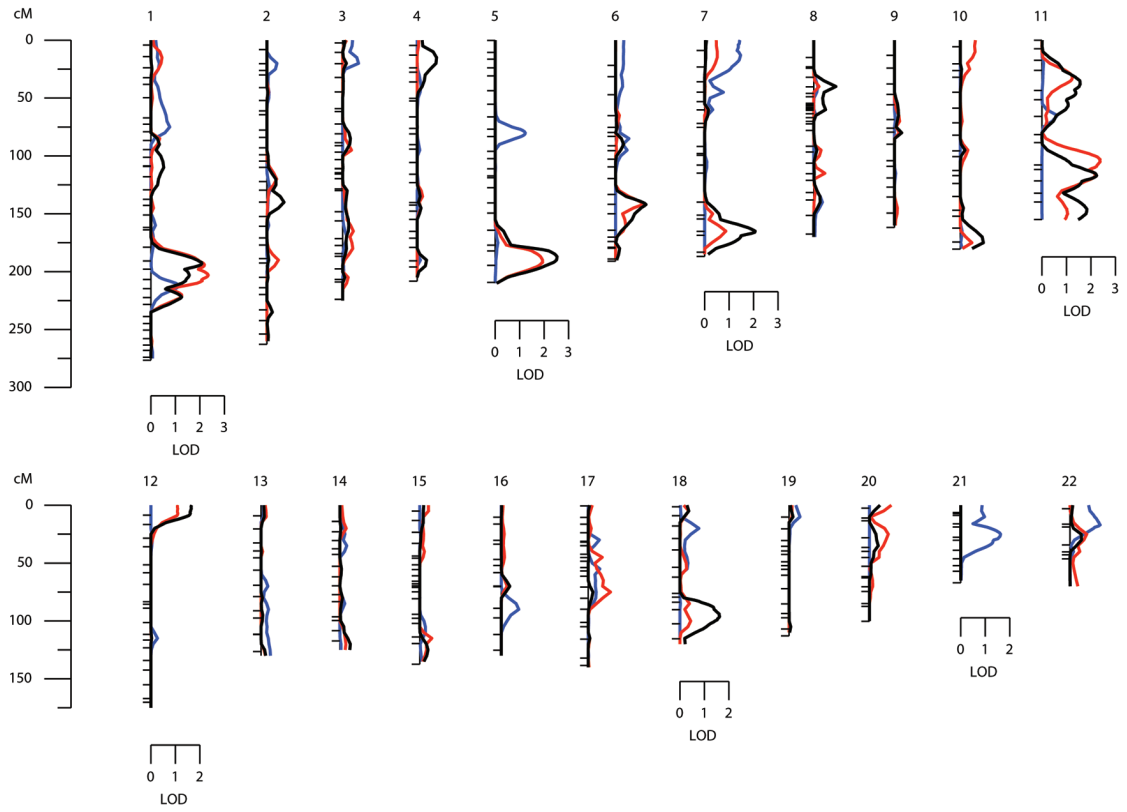

Figure 4. Multipoint linkage analysis of BP traits from clinic visit 2, with use of a 10-cM genome scan of Mexican Americans of the SAFHS. Traits analyzed are SBP (*black*), DBP (*blue*), and MBP (*red*) collected at clinic visit 2. The horizontal scale shows LOD scores >1.5, and the vertical scale shows distance (cM).

and are thus not independent), is suggestive of overall trends in the study sample.

The longitudinal BP genome-scan results are shown in figure 1. We found evidence of linkage that met the criteria for genomewide significance on chromosome 11q24.1 for rSBP (LOD 4.15; $P = .000006$) and for rMBP (LOD 3.94; $P = .00001$) near marker *D11S4464* (table 2 and fig. 2). The same marker also was linked to rDBP (LOD 2.27; $P = .0006$) (table 2 and fig. 2). We identified suggestive linkage of rSBP to the *D16S769* marker on chromosome 16 (LOD 2.73; $P = .0002$) and of rMBP to the *D5S817* marker on chromosome 5 (LOD 2.0; $P = .001$) (table 2). Since we excluded those being treated with antihypertensive medication, we reanalyzed the chromosome 11q longitudinal data for all 817 individuals, including those who were being treated with antihypertensive medication at visit 1 or 2. We incorporated the treatment with antihypertensive medication as well as sex, age at visit 1, and the inverse-normal-transformed rBMI as covariates in the linkage analysis. Results show that including those with extreme pathology and incorporating treatment with antihypertensive medication as a covariate in the analysis actually improved the linkage signal for rMBP (LOD 4.8; $P = .000001$) and for rDBP (LOD 3.2; $P = .00006$), whereas rSBP remained the same (LOD 4.15; $P = .000006$). We also reanalyzed the rSBP linkage result with just sex and age as covariates. The results show that the rate of change in BMI as a covariate in the analysis (rSBP LOD 4.15 with rBMI as a covariate) actually improves our linkage signal for rSBP (LOD 3.97 without rBMI as a covariate). Since we obtained significant evidence of linkage to chromosome 11, we tested for locus heterogeneity at this locus by conducting a multipoint HLOD analysis for rSBP. HLOD results for rSBP were negative for locus heterogeneity, meaning that all of the pedigrees contributing to the QTL in this region show a high probability of linkage.

We adjusted the linkage evidence (LOD score) on chromosome 11q downward by comparison with the empirical distribution of LOD scores in the absence of linkage (using the "LOD adjust" routine in SOLAR (Hlod) with at least 10,500 replicates per trait).[32] We maintained our evidence of linkage to this chromosome 11q24.1 region with an adjusted LOD of 3.96 ($4.15 \times 0.955 = 3.96$) for rSBP and an adjusted LOD of 3.35 ($3.94 \times 0.85 = 3.35$) for rMBP.

The single-time-point BP genome-scan results are shown in figure 3 for clinic visit 1 and in figure 4 for clinic visit 2. We found suggestive evidence of linkage of MBP collected at clinic visit 2 (MBP2) near *D11S2000* (LOD 2.37; $P = .0005$) and *D11S1998* (LOD 2.17; $P = .0008$), which is positioned 5.9 Mb from *D11S4464* and within the 16-

Table 3. Gene-Expression Results for rSBP and rMBP in Chromosome 11q QTLs

BP Change and Gene Symbol	h^2 (P)	Reduction in LOD Score (%)	Regression Coefficient (β)	GeneSniffer Rank[a]	No. of SNPs Tested
rSBP:					
CHEK1	.17 (5×10^{-5})	13.8	.073	1	3
EI24	.25 (2×10^{-8})	5.7	−.078	NA	0
PHLDB1	.19 (4×10^{-7})	4.9	.041	NA	1
rMBP:					
EI24	.25 (2×10^{-8})	9.0	−.059	NA	0
CHEK1	.17 (5×10^{-5})	5.8	.016	1	3
TMEM25	.20 (2×10^{-7})	5.2	.037	NA	0
RPUSD4	.18 (1×10^{-6})	4.8	.10	NA	0

[a] Of 221 genes. NA = not applicable.

Mb 1-LOD–score support interval surrounding the rSBP and rMBP QTLs. We also found suggestive linkage of DBP in visit 2 (DBP2) near *D11S2000* (LOD 2.63; $P = .003$) and of SBP in visit 2 (SBP2) near *D11S1998* (LOD 2.23; $P = .0007$). Suggestive evidence of linkage was also found on chromosome 5 for MBP2, SBP2, and DBP2; on chromosome 1 for MBP2 and SBP2; on chromosome 7 for SBP2; on chromosome 6 for SBP at clinic visit 1 (SBP1); and on chromosome 21 for MBP at clinic visit 1 (MBP1), as outlined in table 2.

Gene-Expression Analysis

We analyzed the lymphocyte transcript–expression levels whose coding loci are within the 1-LOD–score support interval on chromosome 11 (~16 Mb physical distance), to determine the extent to which the transcripts account for the original linkage for rSBP and rMBP. We quantified the expression of a total of 169 transcripts representing 157 (71%) of the 221 genes in our 1-LOD–score support interval. The genes with the highest percentage LOD-score reduction for rSBP and rMBP are shown in table 3. Raw and processed transcript information for the five genes listed in table 3 is available in a txt file (online only), which can be imported into a Microsoft Excel spreadsheet. The expression levels of all genes listed in table 3 are significantly heritable ($P < .00001$). The transcript exerting the largest effect (13.8%) in reducing the rSBP LOD score was *CHEK1* (MIM 603078), whereas *EI24* (MIM 605170) had the largest reduction (9.0%) in the rMBP LOD score (table 3).

Expression levels for *CHEK1* and *EI24* were also the second-most influential transcripts in reducing the LOD scores for rMBP (5.8%) and rSBP (5.7%), respectively (table 3). *CHEK1* was positively correlated with rSBP ($\beta = 0.073$) and rMBP ($\beta = 0.016$), whereas *EI24* was negatively correlated with rSBP ($\beta = -0.078$) and rMBP ($\beta = -0.059$) in our Mexican American families. Of the remaining genes listed in table 3, *PHLDB1* reduced the rSBP LOD score by 4.9%, whereas *TMEM25* and *RPUSD4* reduced the rMBP LOD score by 5.2% and 4.8%, respectively (table 3). *PHLDB1* was positively correlated with rSBP ($\beta = 0.041$), whereas *TMEM25* and *RPUSD4* were positively correlated with rMBP ($\beta = 0.037$ and $\beta = 0.10$, respectively) in our Mexican American families.

Bioinformatic Analysis

We used GeneSniffer to rank the most likely candidate genes in our 16-Mb chromosome 11q region, as shown in tables 3 and 4. Although *CHEK1* has no known involvement in BP regulation, *CHEK1* was identified by GeneSniffer to be the strongest candidate in this region, because it has similarity to the serine/threonine kinase family (Swiss-Prot). CHEK1 therefore belongs to the Ser/Thr protein kinase family NIM1 subfamily (Swiss-Prot). Two members of the serine/threonine kinase family—WNK1 (MIM 605232) and WNK4 (MIM 601844)—were identified in a rare monogenic hypertension syndrome known as "Gordon syndrome," or psuedohypoaldosteronism type 2 (PHA2 [MIM 145260]).[37,38] These *WNK* genes belong to the Ser/Thr protein kinase family WNK subfamily (Swiss-Prot). Both the *CHEK1* and *WNK* genes have a conserved region containing the protein kinase domain. There is ~27% homology between the CHEK1 and WNK proteins within this conserved region.

SNP-Association Analysis

We selected a total of 85 SNPs reported elsewhere from 49 genes identified as potential BP candidates. We selected three SNPs in *CHEK1,* because GeneSniffer ranked it as the strongest candidate gene and its expression level reduced the rSBP and rMBP LOD score by 13.8% and 5.8%, respectively (table 3). We also selected a SNP in *PHLDB1,* because the expression level of this transcript reduced the rSBP LOD score by 4.9% (table 3). In addition, we selected 18 SNPs within 15 genes that we considered likely BP candidate genes that did not have enough information to be ranked by GeneSniffer. The remaining 63 SNPs were positioned in 31 genes that were within the top third of most-likely BP candidates identified by our bioinformatic searching (table 4). The 85 SNPs were genotyped in all 1,400+ Mexican American participants in the SAFHS.

Association analysis shows that SNPs in nine genes had marginal association ($P < .048$) with rDBP, rSBP, or rMBP

Table 4. SNPs Selected in the Potential BP Candidate Genes Positioned within Our 1-LOD–Score Support Interval on Chromosome 11q

Location (Mb)	Gene Symbol	Size (kb)	GeneSniffer Ranking[a]	No. of SNPs		*P*		
				Coding	Tested	rMBP	rDBP	rSBP
111.520	*IL18*	20.9	NA	0	1	NS	NS	NS
112.370	*NCAM1*	315.2	4	0	1	NS	NS	NS
112.786	*DRD2*	65.6	3	0	1	NS	NS	NS
113.281	*HTR3B*	41.7	43	1	1	NS	NS	NS
113.351	*HTR3A*	15.1	23	1	1	NS	NS	NS
116.206	*APOC3*	3.2	19	1	1	NS	NS	NS
116.212	*APOA1*	1.9	2	0	3	NS	NS	NS
116.609	*RNF214*	52.6	NA	0	2	NS	NS	NS
116.804	*DSCAML1*	369.5	52	1	3	NS	NS	NS
117.363	*IL10RA*	15.1	62	1	4	NS	NS	NS
117.453	*TMPRSS4*	41.4	64	1	2	.047	NS	NS
117.509	*SCN4B*	19.4	30	0	1	NS	NS	NS
117.681	*EVA1*	10.9	24	1	2	NS	NS	NS
117.736	*UBE4A*	39.6	NA	0	2	.037	NS	NS
117.812	*MLL*	85.7	NA	1	1	.048	NS	NS
117.982	*PHLDB1*	51.5	NA	1	1	NS	.019	NS
118.126	*DDX6*	41.5	NA	1	1	NS	NS	NS
118.400	*SLC37A4*	6.4	NA	0	1	NS	NS	NS
118.472	*DPAGT1*	5.6	NA	1	1	NS	NS	NS
118.525	*ABCG4*	13.6	NA	0	1	NS	NS	NS
118.684	*MCAM*	8.9	13	1	1	NS	NS	NS
118.715	*C1QTNF5*	1.9	25	1	1	NS	NS	NS
118.794	*THY1*	5.0	14	1	1	NS	NS	.019
119.616	*POU2F3*	79.6	42	1	3	.033	.023	NS
119.782	*ARHGEF12*	83.8	41	0	3	NS	NS	NS
120.36	*GRIK4*	325.9	27	0	6	.018	.029	NS
120.479	*TECTA*	88.1	26	1	1	NS	NS	NS
120.828	*SORL1*	177.5	31	1	3	NS	NS	NS
121.491	*BRCC2*	.9	NA	0	1	NS	NS	NS
122.032	*STS-1*	156.7	8	0	2	NS	NS	NS
122.393	*HSPA8*	4.6	10	0	1	NS	.009	NS
122.448	*ASAM*	123.0	47	0	3	NS	NS	NS
123.007	*SCN3B*	7.0	33	0	1	NS	NS	NS
123.100	*ZNF202*	7.0	NA	1	1	NS	NS	NS
123.491	*LOH11CR2A*	31.5	NA	1	1	NS	NS	NS
123.998	*TBRG1*	11.5	56	0	3	NS	NS	NS
124.010	*SIAE*	38.0	NA	0	1	NS	NS	NS
124.128	*ESAM*	9.1	35	1	1	NS	NS	NS
124.240	*ROBO3*	16.5	28	1	3	NS	NS	.046
124.438	*SLC37A2*	25.9	NA	1	1	NS	NS	NS
124.821	*FEZ1*	50.5	49	0	1	NS	NS	NS
125.002	*CHEK1*	29.3	1	0	3	NS	NS	NS
125.336	*CDON*	102.6	50	1	1	NS	NS	NS
125.638	*SRPR*	5.9	NA	1	1	NS	NS	NS
125.679	*DCPS*	42.0	NA	1	2	NS	NS	NS
125.731	*ST3GAL4*	58.4	61	1	1	NS	NS	NS
125.800	*KIRREL3*	575.9	78	0	2	NS	NS	NS
128.069	*FLI1*	118.3	11	2	4	NS	NS	NS
128.213	*KCNJ1*	29.4	5	0	1	NS	NS	NS
Total				28	85			

NOTE.—Shown is the GeneSniffer ranking of each selected gene and the results from SNP-association analysis. NS = $P > .05$.

[a] Of 221 genes. NA = not applicable.

(table 4). Table 5 also lists the mean values by genotype for rDBP, rSBP, and rMBP for the nine significant SNPs. Although the reported *P* values are nominal and are not adjusted for the multiple tests conducted, our most promising result was found between rDBP and *rs7111598* ($P = .009$), located 2,055 bp from the 3′ UTR for the heat shock protein 8 gene (*HSPA8* [MIM 600816]). Interestingly, GeneSniffer ranked this gene as the 10th-most likely BP candidate in this region (table 4). Although we tested only three intronic SNPs, we did not detect any association between the rates of change in any BP traits with the *CHEK1* gene that GeneSniffer ranked as the most likely

Table 5. Mean Values by Genotype for rSBP, rDBP, and rMBP

Trait and SNP	Chromosome 11 Position (Mb)	Mean Value (SE) by Genotype		
		AA	AB	BB
rSBP:				
rs3741311	117.49	.686 (.24)	.892 (.20)	1.096 (.28)
rs6589663	117.76	1.050 (.20)	.590 (.23)	.132 (.39)
rs629470	117.83	.889 (.21)	.624 (.30)	.358 (.54)
rs498872	117.98	.864 (.21)	.789 (.21)	.716 (.34)
rs1001205	118.80	.799 (.25)	.834 (.20)	.868 (.28)
rs2282537	119.69	.963 (.14)	1.426 (.27)	1.890 (.52)
rs10790400	120.06	1.116 (.24)	.864 (.19)	.613 (.25)
rs7111598	122.43	.875 (.20)	.735 (.24)	.595 (.40)
rs7925879	124.25	1.113 (.19)	1.036 (.14)	.959 (.23)
rDBP:				
rs3741311	117.49	.160 (.20)	−.002 (.15)	−.163 (.17)
rs6589663	117.76	.132 (.15)	−.097 (.17)	−.327 (.29)
rs629470	117.83	.019 (.15)	−.173 (.22)	−.366 (.39)
rs498872	117.98	.177 (.16)	−.087 (.16)	−.350 (.25)
rs1001205	118.80	.130 (.18)	−.109 (.14)	−.348 (.21)
rs2282537	119.69	.042 (.11)	.648 (.20)	1.253 (.39)
rs10790400	120.06	.124 (.18)	.050 (.14)	−.024 (.19)
rs7111598	122.43	.145 (.15)	−.200 (.18)	−.544 (.30)
rs7925879	124.25	.288 (.14)	.126 (.10)	−.035 (.17)
rMBP:				
rs3741311	117.49	.104 (.17)	.317 (.14)	.529 (.20)
rs6589663	117.76	.445 (.14)	.149 (.17)	−.148 (.28)
rs629470	117.83	.322 (.15)	.072 (.22)	−.178 (.39)
rs498872	117.98	.396 (.16)	.238 (.15)	.078 (.25)
rs1001205	118.80	.306 (.18)	.227 (.14)	.147 (.21)
rs2282537	119.69	.372 (.11)	.924 (.19)	1.475 (.38)
rs10790400	120.06	.429 (.18)	.337 (.14)	.244 (.19)
rs7111598	122.43	.377 (.15)	.156 (.18)	−.065 (.30)
rs7925879	124.25	.571 (.14)	.441 (.10)	.311 (.17)

candidate gene in the region. We observed significant association between rDBP and *rs498872* ($P = .019$), positioned within the 5′ UTR of *PHLDB1*; *rs2282537* ($P = .023$), positioned within exon 12 of *POU2F3* (MIM 607394); and *rs10790400* ($P = .029$), positioned within intron 1 of *GRIK4* (MIM 600282). A bioinformatic search of these genes with use of GeneSniffer ranked *GRIK4* and *POU2F3* as the 27th- and 42nd-most likely BP candidate genes in this region (table 4). There was not enough available information for GeneSniffer to rank *PHLDB1*. Interestingly, *rs10790400,* positioned within intron 1 of *GRIK4,* and *rs2282537,* a missense mutation (Lys390Arg) positioned within exon 12 of *POU2F3,* also showed significant association to rMBP ($P = .018$ and $P = .033$, respectively). Significant association was also found between rMBP and *rs6589663* ($P = .037$), positioned within intron 13 of *UBE4A* (MIM 603753); *rs3741311* ($P = .047$), positioned within the 3′ UTR of *TMPRSS4* (MIM 606565); and *rs629470* ($P = .048$), positioned within intron 1 of *MLL* (MIM 159555). GeneSniffer did not rank *UBE4A* and *MLL* but ranked *TMPRSS4* as the 64th-most likely BP candidate in the region (table 4). Only two SNPs—*rs1001205,* positioned within intron 1 of *THY1* (MIM 188230), and *rs7925879,* positioned within intron 6 of *ROBO3* (MIM 608630)—showed nominal significant association to rSBP ($P = .019$ and $P = .046$, respectively). GeneSniffer respectively ranked *THY1* and *ROBO3* as the 14th- and the 28th-most likely BP candidate genes in this region (table 4).

Discussion

The key finding in this study is the identification of QTLs for longitudinal BP measurements on chromosome 11q near marker *D11S4464.* Five other studies also report modest evidence of linkage to BP and/or hypertension on chromosome 11q, consistent with our results. The populations include: U.K. whites,[39] U.S. whites,[40] African Americans from the Hypertension Genetic Epidemiology Network (HyperGen),[41] Hong Kong Chinese,[42] and whites from the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) study.[43] The U.K. population study identified a 44-cM linkage region with peak linkage occurring near marker *D11S934* ($P < .004$) in 169 sib pairs affected with early-onset (aged <50 years) hypertension.[39] Their peak linkage occurred within 3 Mb of our own linkage peak in Mexican Americans. This is also within 24 Mb of a QTL in the HyperGen study, which found a LOD score of 1.23 ($P = .0087$) on chromosome 11q in 169 African American sibships with two or more individuals with hypertension onset before age 35 years.[41] Another study of early-onset hypertension in U.S. whites also identified peak linkage (LOD 1.66; $P = .0029$) within 50 Mb of our QTL region.[40] In Hong Kong Chinese, a LOD score of 1.44 ($P = .005$) was found between *D11S1995* and *D11S1998* for SBP.[42] This linkage peak occurred within 11 Mb of our linkage peak and within 44 Mb of a QTL in the HERITAGE study of whites, where a LOD score of 1.98 ($P = .0013$) near the *D11S2002* marker was identified for resting SBP.[43] Therefore, our linkage to the rate of change of SBP (LOD 4.15) on chromosome 11q replicates the previous findings of linkage to SBP in Hong Kong Chinese[42] and to resting SBP identified in the HERITAGE study of whites[43] in this same chromosome 11q region.

In addition to these results, QTLs in this genomic region have been identified elsewhere for BP-related traits. In Pima Indians, the same marker, *D11S4464,* was linked to BMI and type 2 diabetes, with LOD scores of 3.6 and 1.7, respectively.[44] In the Framingham study, six measures of BMI at different times mapped to the same region.[45] Percentage of body fat and energy expenditure may also be related to the QTL in this region.[46] However, there is evidence that this region of chromosome 11q contains genetic variation affecting hypertension and/or BP independent of obesity and insulin-resistance phenotypes. In the genetic analyses for the HyperGen and HERITAGE studies, incorporation of BMI as a covariate did not significantly affect the evidence of linkage of early-onset hypertension[41] and resting SBP[43] to chromosome 11q. In addition, little of the variation in SBP was explained by waist circumference and insulin resistance, as measured by the HOMA-IR (i.e., homeostasis model assessment of insulin resistance), when incorporated into the linkage analysis of SBP in the Hong

Kong Chinese study.[42] Our results show that the rate of change in BMI as a covariate in the analysis (rSBP LOD 4.15 with rBMI as a covariate) actually improves our linkage signal for rSBP (LOD 3.97 without rBMI as a covariate), implying that changes in weight are not responsible for our QTLs for changes in BP over time.

We also find that the use of measurements of long-term levels of BP provides a better insight into the identification of BP-susceptibility loci, particularly in this chromosome 11q region. This conclusion is based on a comparison of the longitudinal and single-time-point BP genome scans. Significant evidence of linkage is obtained for rSBP and rMBP near the *D11S4464* marker, but only suggestive evidence of linkage of SBP and MBP is found within this region. We attribute the higher LOD scores obtained for rate of change in BP traits to the longitudinal data that provide more information than the single-time measurements, since they reveal physiological trends in the subjects that a single-time measurement could never capture, particularly in this chromosome 11q region. This is further supported by the lack of replication in linkage results between single-time-point BP traits from clinic visit 1 and clinic visit 2. The results showing suggestive linkage to clinic visit 2 BP traits, together with the significant linkage to the rate of change in BP traits, provide further support for a BP-susceptibility locus on chromosome 11q in Mexican Americans of the SAFHS. However, further investigation of this region is necessary to identify the genetic variant(s) responsible for the localization of an age-related BP QTL within the chromosome 11q region.

For this reason, we began identifying the responsible genetic variant(s) by a combination of bioinformatic search of databases, gene expression, and preliminary exploratory SNP-association analyses within the 16-Mb 1-LOD–score support interval of our chromosome 11q QTL. We found agreement in results between the gene-expression and GeneSniffer analyses, because both identified *CHEK1* as the most promising BP-candidate gene in the chromosome 11q QTL region. Although testing three intronic SNPs did not implicate *CHEK1* involvement in any of the change in BP traits, we feel that this gene is our most promising positional candidate in our chromosome 11q BP QTL region. Although SNP analysis identified involvement for a number of other genes in this region, we found little agreement in results among the three different methods for these additional genes. This may occur because the initial SNP panel was too sparse to thoroughly analyze all of the plausible candidate genes in the region. Further investigation may find linkage disequilibrium between the SNPs in this study and those yet to be genotyped in genes identified by GeneSniffer and gene-expression analysis.

Some would also argue that the lack of consistency among the three fine-mapping approaches for these additional genes occurred because gene expression in lymphocytes is not representative of gene expression in tissues relevant to hypertension. Previous studies show that lymphocytes express a number of BP-related genes—including angiotensin 1–converting enzyme,[47] angiotensin II, and renin[48]—and several receptors—including cytokines, glucocorticoids, catecholamines, dopamine, and acetylcholine.[49] Examination of gene-expression levels in lymphocytes may also detect other genes (such as *CHEK1*) that normally are not considered to have involvement in BP regulation. For these reasons, we believe lymphocyte-expression studies to be quite valuable for etiological studies.

We conclude that a more thorough SNP investigation of *CHEK1* may identify the responsible genetic variant(s). This, in turn, will provide further proof that a combination of GeneSniffer, gene-expression, and SNP-association analyses is the most effective approach for the identification of positional candidate genes contributing to our chromosome 11q change in BP QTLs in Mexican Americans of the SAFHS. Genetic variation that is ultimately identified as affecting the regulation of BP over time may not be confined to Mexican Americans, and studies of other populations that include a simple longitudinal recording of an individual's BP measurement along with screening for the existence of the identified variant(s) will confirm this phenomenon.

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Web Resources

The URLs for data presented herein are as follows:

Entrez Gene, http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
GeneSniffer, http://www.genesniffer.org/
Hlod, http://www.sfbr.org/solar/doc/95.appendix_5.txt
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *CHEK1*, *EI24*, WNK1, WNK4, PHA2, *HSPA8*, *POU2F3*, *GRIK4*, *UBE4A*, *TMPRSS4*, *MLL*, *THY1*, and *ROBO3*)
Swiss-Prot, http://us.expasy.org/uniprot/O14757

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