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A Genome Scan for Hypertension Susceptibility Loci in Populations of Chinese and Japanese Origins

Koustubh Ranade, David Hinds, Chao Agnes Hsiung, Lee-Ming Chuang, Mau-Song Chang, Ying-Tsung Chen, Robert Pesich, Joan Hebert, Yii-Der I. Chen, Victor Dzau, Richard Olshen, David Curb, David Botstein, David R. Cox, and Neil Risch

Background: Our understanding of genes that predispose to essential hypertension is poor.

Methods: A genome-wide scan for linkage at ~10 cM resolution was done on 1425 sibpairs of Chinese and Japanese origins that were concordant for hypertension (N = 661), low–normal blood pressure (BP) (N = 184), or discordant for BP (N = 580).

Results: There was no significant evidence of linkage to a single locus in the genome. There was suggestive evidence of linkage to chromosome 10p, with a LOD score of 2.5.

Conclusions: We can exclude the possibility that a single gene accounts for at least 15% of the variance in hypertension in this population. Am J Hypertens 2003;16:158–162 © 2003 American Journal of Hypertension, Ltd.

Key Words: Hypertension, genetics, linkage.

Despite intense effort, our understanding of heritable factors in the etiology of essential hypertension is rather poor. Twin, adoption, and epidemiologic studies indicate that variation in blood pressure (BP) is genetically determined to some extent (for a recent review, see Ref. 1). One of the goals of the Stanford, Asia and Pacific Program for Hypertension and Insulin Resistance (SAPPHIRe) is to identify genes for essential hypertension. To maximize our chances of finding such genes, we took several steps. First, in an attempt to reduce genetic heterogeneity, we focused on relatively homogeneous populations of Chinese descent recruited from Taiwan, the San Francisco Bay area, and Hawaii, and of Japanese origin recruited in the San Francisco Bay area and Hawaii. Second, we sampled subjects from tails of the BP distribution—the affected hypertensives were recruited from the upper 20% of the distribution, whereas the unaffected "low–normotensive" individuals had BP readings in the lower 30% of the distribution. Furthermore, in addition to BP we collected data on a large number of metabolic variables, such as plasma glucose, insulin, triglycerides, and cholesterol, in the belief that the contribution of individual genes might be more apparent for these "proximate" phenotypes than for a "distant" phenotype like hypertension.

Here we report results for a 10-cM genome scan for hypertension susceptibility loci using more than 1400 sibpairs of Chinese and Japanese origins.

Methods

Subjects

The study populations and inclusion/exclusion criteria have been previously described in detail.[2] Briefly, the study design incorporated both concordant sibpairs (at

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From the Department of Genetics (KR, DH, RP, JH, DB, DRC, NR) Stanford University School of Medicine, Stanford, California; National Health Research Institute (CAH), Taipei, Taiwan; Department of Internal Medicine (L-MC), National Taiwan University Hospital, Taipei, Taiwan; Department of Internal Medicine (M-SC), Taipei Veterans General Hospital, Taipei, Taiwan; Department of Internal Medicine (Y-TC), Taichung Veterans General Hospital, Taichung, Taiwan; Division of Endocrinology (Y-DIC), Stanford University School of Medicine, Stanford, California; Department of Medicine (VD), Brigham & Women's Hospital, Boston, Massachusetts; Health Research and Policy (RO), Stanford University School of Medicine, Stanford, California; and Hawaii Center for Health Research (DC), Honolulu, Hawaii.

Dr. Ranade's present address is Pharmaceutical Research Institute, Bristol-Myers Squibb, Princeton, NJ 08543-5400.

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Address correspondence and reprint requests to: Koustubh Ranade, Pharmaceutical Research Institute, Bristol-Myers Squibb, PO Box 5400, Princeton, NJ 08543-5400; e-mail: koustubh.ranade@bms.com

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least two sibs with hypertension) and discordant sibpairs (at least one hypertensive and one with low–normal BP). From these families, we also derive sibpairs concordant for low–normal BP. Such pairs are always the sibs of a hypertensive proband. Hypertension was defined as follows: systolic blood pressure (SBP) ≥160 mm Hg or diastolic blood pressure (DBP) ≥95 mm Hg or taking two medications for high BP (stage II hypertension). Alternatively, the subject had uncontrolled hypertension, ie, was taking one medication for high BP and had either systolic BP ≥140 or diastolic ≥90 mm Hg. Low–normal BP was defined as BP in the bottom 30% of the age- and sex-adjusted BP distribution, which in our populations translate into the following BP values. For men less than 45 years, SBP ≤115 mm Hg and DBP ≤76 mm Hg. For men more than 45 years, SBP ≤122 and DBP ≤78 mm Hg were used. For women younger than 45 years, low–normal BP was defined as SBP ≤107 and DBP ≤70 mm Hg. For those more than 45 years, the cutoff was SBP ≤118 and DBP ≤75 mm Hg. There was no upper age cutoff for sibs with low–normal BP as long as both SBP and DBP readings were below the limit. However, the sib with low–normal BP had to be ≥35 years.

For the current study, 1425 sibpairs were genotyped. Of these, 1123 are of Chinese descent and the remainder are Japanese. There were 661 and 221 sibpairs of Chinese and Japanese origin, respectively, that were concordant for hypertension. One hundred eighty-four pairs, of which 167 were Chinese, were concordant for low–normal BP. There were 580 sibpairs, of which 516 were Chinese, that were discordant for hypertension. Note that these sibpairs are not all independent. For example, a family with two hypertensive sibs and two sibs with low–normal BP provided six sibpairs—one pair each concordant for hypertension or low–normal BP and four discordant pairs.

This study was approved by Institutional Review Boards at all participating sites and all subjects gave written informed consent.

BP Measurements

Blood pressure was measured with an oscillometric device, the Dinamap model 1846 SX (Critikon Inc., Tampa, FL) at all field centers, using the following protocol. The participant was seated with both legs uncrossed and asked to refrain from talking for 5 min. After determining the proper cuff size, BP measurements were taken three times with at least 1-min time lapse between two readings, and the average of the second and third readings was used in the analysis. To ensure uniform BP measurement at the different sites, technicians/clinicians at all the sites were trained to measure BP using this protocol, and they were monitored annually during site visits. Furthermore, there was a centralized retraining and recertification of key technicians each year.

Genotyping

All genotyping was done by the Marshfield Medical Research Foundation. Most individuals were typed for 388 markers from Screening Set 9 (http://www.marshmed.org/genetics)[3] to yield ~10-cM map. Genotyping quality was monitored by typing 30 samples in duplicate. We estimate an error rate of ~1% based on these duplicate samples.

Linkage Analysis

We used the ASPEX package (ftp://lahmed.stanford.edu/pub/aspex/) to screen the data for non-Mendelian segregation (eg, nonpaternity) and subsequently for nonparametric linkage analysis of the sibpair data. Using the "sib_kin" program in ASPEX, we identified 24 families with one or more nonpaternities, 8 pairs of identical twins, and 1 sample mixup (a sib identical to a parent). These families and individuals were excluded from the linkage analysis. Data for three markers, which had significant Mendelian inconsistencies (>1% of the genotypes), were excluded from linkage analysis. For linkage analysis using the "sib_phase" program, we used a published sex-averaged genetic linkage map,[4] and calculated maximum likelihood scores (LOD) under a model with no dominance variance. In multiplex families, all distinct pairs of hypertensive sibs or sibs with low–normal BP were scored equally, without weighting based on family size. Allele frequencies were estimated by counting all individuals, including parents and sibs. The LOD scores for the three types of sibships were combined by converting the LOD scores to Z-scores and then taking a weighted sum of these scores (the number of sibships in that particular category was used as the weight). For the sake of consistency, these summed Z-scores were converted to LOD scores and then plotted (Fig. 1, Supplementary Information; https://www-hrpdcc.stanford.edu/sapphire).

Results

Two thousand four hundred sixty-two individuals were typed for more than 380 markers spread every 10 cM across the genome. These individuals consisted of 661 sibpairs that were concordant for hypertension, 184 pairs that were concordant for low–normal BP, and 580 that were discordant for the phenotype. Of these 1425 sibpairs, 1123 (79%) were of Chinese origin and the remainder were Japanese–Americans. The results for the entire genome scan are posted on the Stanford web site (Supplementary Information, Fig. 1). No single region of the genome passed the genome-wide significant LOD score threshold of 3.6 for any of the three types of sibpairs, either individually or summed across the different types of sibpairs.

Only one region of the genome showed consistent sharing for all three types of sibpairs and provided suggestive evidence of linkage, with a LOD score of ~2.5. Markers on chromosome 10, ~30 cM from the telomere of the p arm (flanking markers are D10S189 and D10S1423) showed less than 50% sharing for the discordant sibpairs

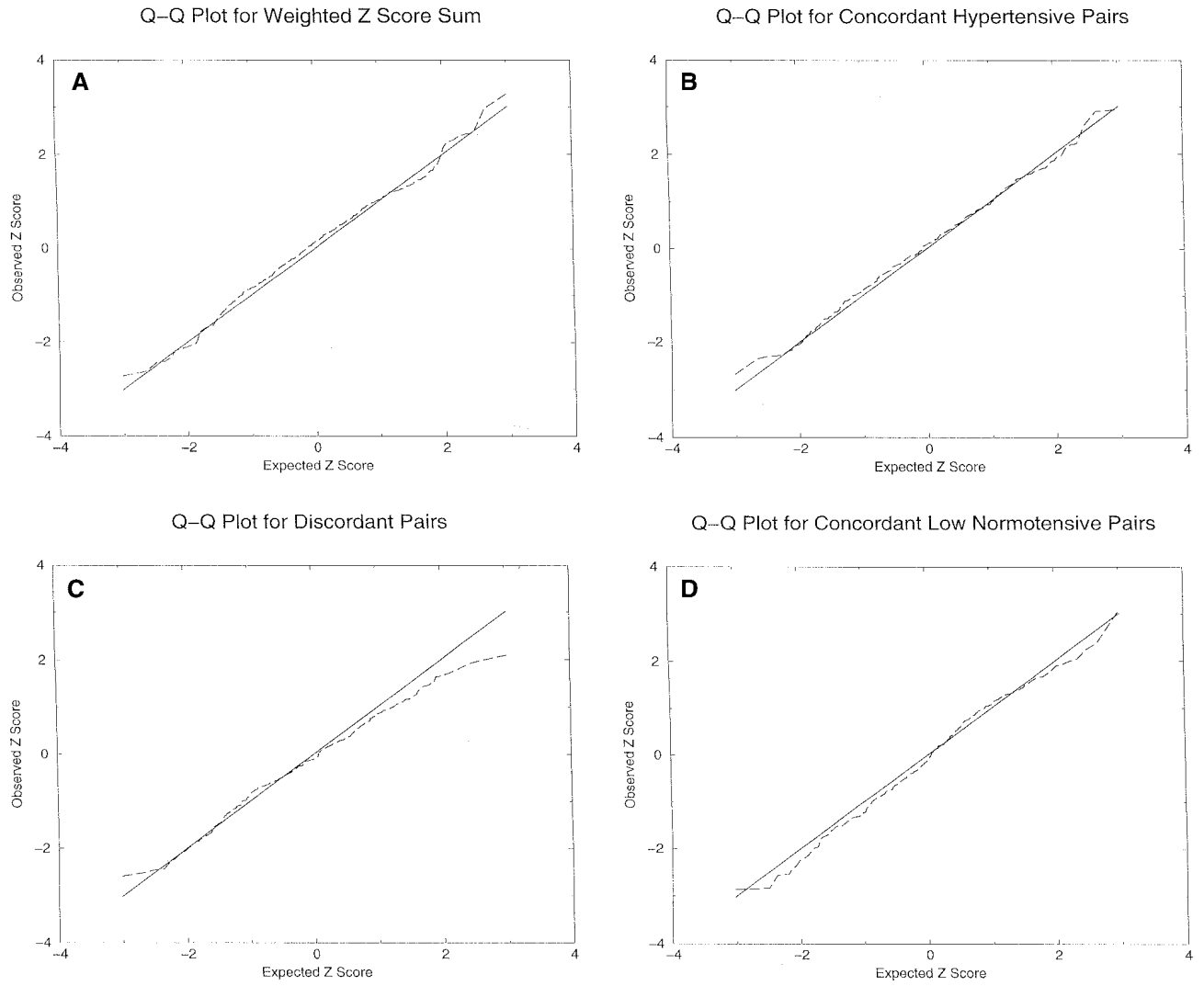

FIG. 1. Q–Q plots for genome-wide allele sharing. The observed Z-scores (y-axis) are plotted against the expected Z-scores (x-axis). Under the null hypothesis, this plot should be a **straight line** (**solid line** in all panels). **Panels A, B, C,** and **D** show the distributions of Z-scores for all three types of sibpairs combined, concordant hypertensive sibpairs, discordant sibpairs, and sibpairs concordant for low–normal blood pressure.

and produced a LOD score of 1.5. Both types of concordant sibpairs showed more than 50% sharing in this region of chromosome 10 and produced LOD, scores of ~0.7. However, the composite LOD score of 2.5 in this region is still well below the genome-wide significance level.[5]

Two other regions of the genome produced a LOD score of ~2. Sibpairs concordant for low–normal BP showed more than 50% sharing for markers near the end of the q arm of chromosome 9, resulting in a LOD score of 2 (nearest marker is D9S1838). Sibpairs concordant for hypertension, on the other hand, produced a LOD score of 2, ~100 cM from the telomere of the p arm of chromosome 14 (solid line, chromosome 14 panel, flanking markers D14S606 and GATA30A03). However, neither region showed consistently excess sharing (among the concordant sibpairs) or less than 50% sharing among the discordant sibpairs, thus the composite LOD scores in these regions were well below 2. Numerous other regions of the genome produced LOD scores of ~1, as would be expected merely by chance.[5]

We also analyzed the distribution of genome-wide allele sharing for the different types of sibpairs, essentially as described.[6] These results are shown in Fig. 1. For each marker, we calculated a Z-score as $2.146 \times (\text{LOD})^{1/2}$, where LOD is the multipoint LOD score; a minus sign was affixed if the allele sharing at that location was estimated to be less than 50%. The Z-scores were then plotted as a function of $\Phi^{-1}[(R - 1/2)/S]$, where R was the ordered rank of that Z-score, S was the total number of markers (388), and Φ was the cumulative normal distribution. Under the null hypothesis of no linkage, this curve should fit a straight line y = x. In Fig. 1, the line y = x is depicted

as the solid line. As can be seen in Fig. 1A, the line for the composite score (dashed line) runs almost parallel to the line y = x, but is displaced from it, with significant deviations at either end. The deviation at the right end of the line, suggests slightly more sharing than expected, and corresponds to loci on chromosome 10. The deviation at the left suggests a slight deficiency of negative sharing (ie, less than 50% sharing). This pattern is largely reproduced in the other types of sibpairs (Fig. 1B, C, and D). These observations are consistent with loci of small effect segregating in our population.

Discussion

We did not find significant evidence for a single susceptibility locus for hypertension. The results we obtained are directly comparable to those from another study[7] that also used Chinese subjects and a study design—sibpairs concordant and discordant for BP—similar to the one used here. However, we found little or no evidence of linkage for loci that were implicated in that study. In contrast to LOD scores greater than 2 seen on chromosomes 3, 11, 15, 16, and 17 in that study, we found peak LOD scores well under 1 in these regions. This inconsistency in results in populations of the same ethnicity and ascertained using similar criteria might be due to extreme heterogeneity. Alternatively, it might be that the original finding was a false positive. We also compared our results with those obtained from a large genome scan for loci contributing to variation in SBP in a population of white Americans.[8] The region on chromosome 6 that showed the most significant evidence for linkage in that study produced a composite LOD score of ~0.3 in our population. Furthermore, in contrast to three studies that found evidence for linkage on chromosome 17,[9–11] we find little evidence for linkage to any region of chromosome 17.

How does one reconcile the fact that although a substantial proportion of BP variation and susceptibility to essential hypertension are genetic, several genome scans with large sample sizes failed to find consistently significant evidence for linkage? As shown by the Fig., the most likely answer is the low heritability or displacement for any single contributing locus.[12] For instance, in this study, although we typed more than 1400 sibpairs, we had only sufficient power to exclude the hypothesis that a single causative gene accounted for ~15% of the variance in hypertension. We note that this study was designed to be a part of the Family Blood Pressure Program (see accompanying articles). Thus, although our study had sufficient power to detect a gene that explained 15% (or greater) of the variance in hypertension, the program as a whole had power to detect genes of much smaller effect. If, however, susceptibility to hypertension is determined by three or even two genes acting independently, with each one having only a modest effect, we would, in general, have failed to detect them. Alternatively, it is possible that common alleles of many different genes conspire to make an individual susceptible to hypertension. Under this polygenic model, each such allele would account for only a small proportion of the variance in BP; again, we would not have detected such genes. Theoretical work suggests that, using linkage analysis, one would require a large number of sibpairs to detect loci of this type.[13] Association studies with defined variants in candidate genes and with sample sizes such as ours, on the other hand, were found to be adequately powerful.

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