Original article

Linkage analysis of chromosome 14 and essential hypertension in Chinese population

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Background Hypertension is a complex biological trait that influenced by multiple factors. The encouraging results for hypertension research showed that the linkage analysis can be used to replicate other studies and discover new genetic risk factors. Previous studies linked human chromosome 14 to essential hypertension or blood pressure traits. With a Chinese population, we tried to replicate these findings.

Methods A linkage scan was performed on chromosome 14 with 14-microsatellite markers with a density of about 10 centi Morgen (cM) in 147 Chinese hypertensive nuclear families. Multipoint non-parametric linkage analysis and exclusion mapping were performed with the GENEHUNTER software, whereas quantitative analysis was performed with the variance component method integrated in the SOLAR package.

Results In the qualitative analysis, the highest non-parametric linkage score is 1.0 ($P=0.14$) at D14S261 in the single point analysis, and no loci achieved non-parametric linkage score more than 1.0 in the multipoint analysis. Maximum-likelihood mapping showed no significant results, either. Subsequently the traditional exclusion criteria of the log-of-the-odds score-2 were adopted, and the chromosome 14 with $\lambda s \geqslant 2.4$ was excluded. In the quantitative analysis of blood pressure with the SOLAR software, two-point analysis and multipoint analysis suggested no evidence for linkage occurred on chromosome 14 for systolic and diastolic blood pressure.

Conclusion There was no substantial evidence to support the linkage of chromosome 14 and essential hypertension or blood pressure trait in Chinese hypertensive subjects in this study.

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Human hypertension is a complex biological trait that influenced by multiple environmental and genetic factors and their interactions. Important categories of environmental exposures have been identified, but genetic architecture of this trait remains obscure.[1] Blood pressure (BP) has presented a particularly difficult challenge to genetic epidemiology. As is generally recognized, there are likely to be multiple quantitative trait loci (QTLs) involved in BP regulation in the general population, each with small-to-moderate effects. Of course, genetic effects are likely to be unevenly distributed among loci, and specific genomic regions could be relatively important in different populations.[2] One of the important tasks of hypertension research is the elucidation of the genetic basis of hypertension as a means to identify the genes involved, determine their respective role in causing high blood pressure, and establish how they interact with one another and with non-genetic factors to result in the hypertensive phenotype.[3] One research method that has been available so far for achieving the aim is linkage analysis. The encouraging results[4] for hypertension research demonstrated that despite past disappointments, linkage studies can be used

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to replicate regions from other studies and potentially discover new genetic risk factors of moderate to large effect size.

Among the linkage findings for hypertension and BP variations, chromosome 14 has been shown interesting in a number of studies. Wowern's study[5] in Scandinavian sib-pairs suggested the region near the marker D14S288 [around 41 centi Morgen (cM)] to be the susceptibility locus for early onset primary hypertension [log-of-the-odds score (LOD score) 2.7, $P=0.0002$]. The nominal evidence of linkage was reported around marker D14S306 (LOD score 0.97, $P<0.05$), at 44 cM for diastolic blood pressure (DBP) in a genome scan of Mexican American families.[6] In the genome-wide linkage analysis of Quebec family study,[7] suggestive evidence for systolic blood pressure (SBP) was found on the region 14q, revealing a LOD score of 1.43 ($P=0.00514$) at marker D14S283 for combined population, and a LOD score of 1.37 ($P=0.00599$) at marker D14S1280 for random population. All three studies suggested chromosome 14 as a possible target region for linkage analysis, but enormous discrepancies exist in the different studies.[8-17] Therefore, more studies are needed to verify the results claimed. In this study, we scanned chromosome 14 with microsatellite markers of 10 cM intervals in 147 Chinese hypertensive nuclear families.

METHODS

Study population

We recruited 147 hypertensive families including 799 individuals. All subjects were Han ethnicity, which accounts for 96% of the total population in the mainland of China. Genotypes of all familial members were verified for Mendelian segregation. To be eligible for our study, members of each nuclear hypertensive family had to meet the following criteria: (1) age greater than 15 years; (2) self-identified as having four Han Chinese grandparents; (3) either one of the parents with hypertension; (4) two or more siblings with hypertension; (5) resting-sitting SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg on three different occasions, or taking medications for high blood pressure and having SBP < 140 mmHg and DBP < 90 mmHg; (6) no clinical or biochemical signs of secondary hypertension.

Once families were identified by an initial rigorous screening survey, a field team, which included epidemiologists and cardiologists from Fuwai Hospital was sent to the local site to confirm the family eligibility. After positive confirmation, a formal study was conducted at the local hospital. Standard questionnaires were administered by trained interviewers to collect demographic characteristics, history of cardiovascular disease, family history of hypertension and life-style factors, such as cigarette smoking, alcohol consumption, dietary behavior and physical activity. Anthropometric measurements, including height, weight, waist and hip circumferences, and an electrocardiogram, were taken according to standard protocols. Doctors or nurses at all sites were trained to measure blood pressure using the following protocol. The patient was in the seated position with both legs uncrossed for a 10-minute rest before the blood pressure measurements were taken. After determining the proper cuff size, blood pressure was taken on the subject's right arm with a standard mercury sphygmomanometer after a 10-minute rest. Three measurements were taken with an interval of more than 30 seconds between readings. The study was approved by the Local Research Ethics Committee of the Cardiovascular Institute and Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, and informed consent was obtained from all participants.

Genotyping

Genomic DNA was obtained from peripheral blood leukocytes by standard phenol/chloroform extraction,[18] and the samples were genotyped at the Chinese National Human Genome Centre, Beijing, China.

The primer sets of the microsatellite markers we genotyped were from ABI PRISM™ linkage mapping set (ABI, California, USA). Touch-down polymerase chain reactions (PCR) were performed on 25 ng genomic DNA and 0.5 pmol of each primer with 0.2 U AmpliTaq Gold™ DNA polymerase (ABI, USA). Thermo cycling was performed on ABI GeneAmp® PCR system 9700 (ABI, USA) and cycling parameters consisted of 95℃ for 10 minutes, followed by 35 cycles at 94℃ for 30 seconds, 55℃ for 40 seconds and 72℃ for 1 minute, with the annealing temperature decreasing from 62℃ to 55℃ by 0.5℃ per cycle during the first 15 cycles.

PCR products, DI formamide (Sigma, Texas,

USA), and fluorescent DNA size standard (ABI GENESCAN Rox-400HD) were mixed with blue dextran, and then loaded on a 36-cm, 4.5 denaturing polyacrylamide gel. After electrophoresis on an ABI PRISM™ 377 Sequencer (ABI, USA) in 1 × Tris-borate-EDTA buffer (TBE) at 51℃, 3 kV for 2.5 hours, we performed size-calling using GeneScan® 3.1 and Genotyper® 2.1 software (ABI, USA). Two control samples from CEPH families 1347-02 were used in every plate of samples amplified and on every gel to perform quality control for PCR and electrophoresis.

Statistical analysis

Descriptive statistics mean ± standard deviation (SD) were shown to evaluate the characteristics of all participants. Student's *t* test was used to compare subjects by age, body mass index (BMI), SBP, and DBP with SAS program. A *P* value less than 0.05 was considered statistically significant. We applied both qualitative trait analysis and quantitative analysis to perform the statistical analysis. The covariation evaluated included age, sex, and the interaction of age and sex.

Qualitative analysis

We used the GENEHUNTER program (GENEHUNTER 2.0 package, Whitehead Institute, Cambridge, Massachusetts, USA) to perform the qualitative (i.e. the affected status) mapping via decomposing a pedigree into pairs of relatives. In a model-free multipoint non-parametric-linkage (NPL) method, inheritance information from all markers can be extracted and all pedigree members can be taken into account. Both single point and multipoint analysis were used to assess evidence for linkage.[19] Exclusion mapping and maximum-likelihood (ML) mapping were performed under the multipoint scheme.

NPL analysis first computes the allele sharing proportions at each location along the chromosome, plotted as Z_0, Z_1 and Z_2, given by: $z_0 = a_0/\lambda s$, $z_1 = a_1$, $z_2 = a_2((2\lambda s - 1)/\lambda s)$, where λs is the relative risk ratio for a sib, $a_0 = 1/4$, $a_1 = 1/2$, and $a_2 = 1/4$. A maximum LOD score Z (i.e. NPL score) is then computed at each location, comparing the likelihood of the observed data arising under the estimated sharing proportions with the likelihood under random Mendelian segregation: $L(pos) = (z_0 \times p_0 + z_1 \times p_1 + z_2 \times p_2)/(a_0 \times p_0 + a_1 \times p_1 + a_2 \times p_2)$. And the LOD score is calculated by summing $\log_{10}(L(pos))$ across pedigrees for each position. Exclusion was declared at a LOD score < -2. Likelihood is maximized under the assumption of no dominance variance (i.e. z_1 is fixed at 0.5).[20]

Quantitative analysis

The variance component method is widely used in quantitative genetics and is typified by the SOLAR package (SOLAR 1.7.4 package, sequential oligogenic linkage analysis routines; Southwest Foundation for Biomedical Research, San Antonio, Texas, USA). This method that did not require detailed specification of the mode of inheritance was used to determine linkage of SBP levels and DBP levels with genetic markers. The proportion of marker alleles shared identical by descent (IBD) among all relative pairs was estimated. Linkage was assessed by fitting a polygenic model that did not incorporate genetic marker information (i.e. IBD status) and comparing it with models that incorporated genotype data at a specific marker (two-point analysis) or across a chromosome (multipoint analysis). Two-point and multipoint quantitative trait linkage analyses were conducted on the standardized residuals for SBP and DBP. The log (base 10) of the ratio of the likelihoods of the polygenic and marker-specific models is LOD score, the traditional measure of genetic linkage.

RESULTS

A total of 799 individuals from 147 nuclear families were genotyped for 14 markers spread every 10 cM across the chromosome 14. General characteristics

Table. General characteristics of 799 subjects (mean ± SD)

	Parents			Offspring		
	Affected (*n* = 181)	Un-affected (*n* = 115)	*P* value	Affected (*n* = 354)	Un-affected (*n* = 149)	*P* value
Age (years)	72.35 ± 7.53	73.59 ± 9.87	0.166	47.26 ± 8.70	42.08 ± 7.97	< 0.001
BMI (kg/m^2)	23.14 ± 8.36	22.83 ± 2.98	0.169	26.25 ± 4.19	25.37 ± 3.05	0.087
SBP (mmHg)	165.02 ± 23.58	135.56 ± 27.50	0.002	147.84 ± 20.50	120.33 ± 21.16	< 0.001
DBP (mmHg)	86.73 ± 13.09	73.67 ± 8.20	0.003	94.93 ± 11.93	76.87 ± 13.11	< 0.001

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

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of all subjects were shown in Table. In parents as well as in offspring, BMI, SBP and DBP were higher in affected subjects than in un-affected subjects.

Qualitative trait analysis showed that there was no significant evidence for linkage on chromosome 14 with essential hypertension. The two-point NPL analysis using GENEHUNTER software showed the peak NPL score of 1.01 (P = 0.14) at marker D14S261. The multipoint analysis revealed the peak NPL score of 0.10 at marker D14S261 (P=0.45), shown in Fig. 1. Only a little deviation from the null values of 0.25 was shown in ML mapping (Fig. 2).

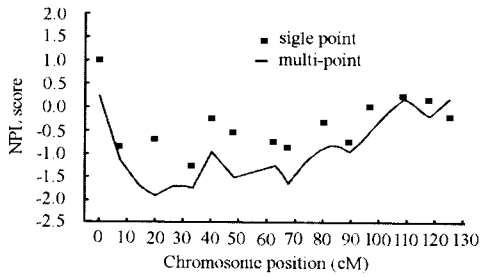

Fig. 1. Qualitative trait analysis using GENEHUNTER 2.0 software.

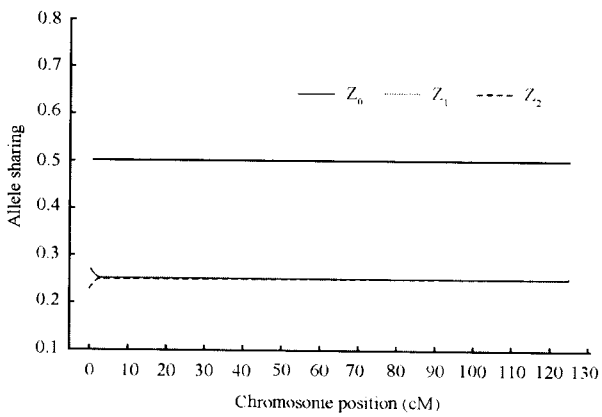

Fig. 2. Maximum-likelihood mapping.

An exclusion map was given in Fig. 3, LOD scores were computed under the assumptions of no dominance variance. For the hypotheses λs = 1.2, 1.5, 1.8, 2.0, and 2.4, the regions proportion excluded were 0, 66%, 84%, 98% and 100%. According to the traditional criteria (LOD score < −2), the whole chromosome was ruled out when λs≥2.4.

SOLAR 1.7.4 package was used in analyzing SBP and DBP with the variance component method. Two

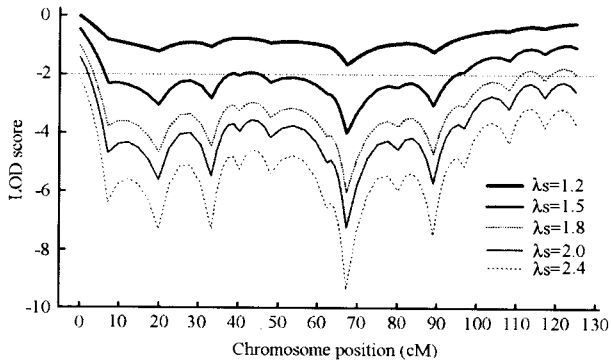

Fig. 3. The exclusion map for chromosome 14.

point linkage analysis showed the highest LOD score of 1.14 at marker D14S74 (80.2 cM) for DBP, and 0.73 at D14S63 (62.3 cM) for SBP. The multipoint analysis revealed no linkage between the markers and DBP or SBP. In Fig. 4, the LOD scores plotted against the location on the chromosome 14, and the highest LOD score was 0.94 at 80 cM and 0.60 at 74 cM for DBP and SBP, respectively.

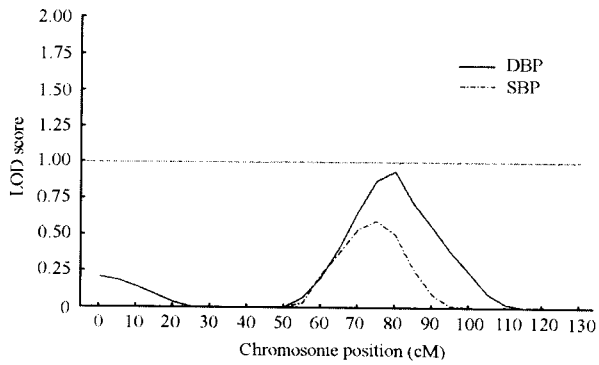

Fig. 4. Multipoint linkage analysis for blood pressure by SOLAR software.

DISCUSSION

The major finding of this study is to exclude chromosome 14 for the linkage with essential hypertension in Chinese population. In screening the genome, one may wish to identify and exclude the regions unlikely to have a major effect on the trait, and then focus attention on the remainder of the genome.[20] Such exclusion mapping is easily performed by GENEHUNTER version 2.0 software. In examining susceptibility loci for systemic lupus erythematosus (SLE), exclusion mapping ruled out linkage at chr14q21-23.[21] In Sharma's study[11] searching for susceptibility loci to human essential hypertension, chromosome 11 was completely

excluded because it exhibits λs = 2.4. In present study, we exclude chromosome 14 in Chinese hypertensive subjects.

Although some reports linked chromosome 14 to hypertension or blood pressure, a number of findings did not support these findings. To date, there are four genome scan for essential hypertension and blood pressure in Chinese population. Xu et al[22] scanned the entire autosomal genome for blood pressure in 564 extreme sib pairs by using 367 polymorphic markers. Although no regions achieved a 5% genome-wide significance level, maximum LOD-score values were >2.0 (unjusted $P<0.001$) for regions containing five markers (D3S2387, D11S2019, D15S657, D16S3396, and D17S1303). Later, Zhu et al[23] carried a three-stage genome-wide linkage study of hypertension in 283 hypertensive sib-pairs with 240 microsatellite marker loci. The results suggested a region on human chromosome 2 that harbors genes contributing to the occurrence of hypertension in the Chinese population. A genome scan was reported by Ranade et al[24] at about 10 cM resolution for hypertension susceptibility loci using 1425 sib-pairs. There was suggestive evidence of linkage to chromosome 10p, with a LOD score of 2.5. Recently, in a large kindred (n = 387) and additional 32 nuclear families, Gong et al[25] performed a genome-wide linkage analysis for essential hypertension using 387 polymorphic microsatellite markers, a new locus on chromosome 12p (parametric LOD score 3.44) was identified. All of above researches did not identify any promising regions on chromosome 14 for essential hypertension or blood pressure.

There are several possible explanations as to why no linkage was detected in this study. Firstly, different heritabilities and family structures between our group and others impact different power to detect genetic effects, as well as uneven distribution of alleles existed in the different populations, assuming that genetic effects are present. Secondly, environmental factors interacting with the influential genes may exist and these factors may be more prevalent in other ethnic groups than in ours. Finally, it may be that the prior results were false-positive because estimates of locus-specific effect size at genome-wide LOD score peaks tend to be grossly inflated.[26]

Large samples of subjects residing in rural areas were used in our study, where the rate of awareness and treatment of hypertension were low.[27] Most hypertensive patients did not take anti-hypertensive drugs, and some patients took them irregularly. The patients were excluded from this study if they had taken medication in the past two weeks and could not be re-examined without withdrawing treatment for two weeks. This enabled us to observe the natural variation of quantitative blood pressure phenotype. So the population was appropriate for collecting hypertensive families for the study of hypertension and quantitative traits in BP.[28] We used NPL method to perform the qualitative analysis. Its efficient use of all available information from simultaneous consideration of both all relatives and all markers makes it to be the method of choice for pedigree studies of complex traits like essential hypertension.[19] For quantitative trait analysis, variance component approach[29] has been found to be powerful and provide more precise estimates of location.

In our study, we observed the highest NPL score at marker D14S261 with both single point and multipoint qualitative analysis method, and ML mapping results also showed a little deviation from segregation at this locus. Indeed, even this weak relation with this locus must be viewed with caution, as the position is at the end of the chromosome, a region where linkage computer program can occasionally generate misleading evidence.[30] In conclusion, our results showed no substantial evidence to support the linkage of chromosome 14 with essential hypertension or blood pressure quantitative trait in our Chinese samples.

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