ELECTRONIC LETTER

Evidence for linkage and association of the markers near the *LPL* gene with hypertension in Chinese families

W J Yang, J F Huang, C L Yao, Z J Fan, D L Ge, W Q Gan, G Y Huang, R T Hui, Y Shen, B Q Qiang, D F Gu

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Essential hypertension (EH) is the most common risk factor for cardiovascular, cerebrovascular, and renal diseases. It is a complex trait that is heritable and involves multiple quantitative trait loci (QTL) and environmental conditions affecting the underlying physiological mechanisms.[1] Genetic linkage studies and genome wide scans have disclosed many possible candidate loci contributing to hypertension.

Hypertension has been found to occur more often than expected in families with familial hyperlipidaemia. Because dyslipidaemia is a common finding in hypertensive patients, the lipoprotein lipase (*LPL*) gene is a logical candidate gene that could contribute to the development of hypertension.[2] LPL is a crucial enzyme in plasma lipoprotein metabolism, which hydrolyses triglycerides and chylomicrons. Two genetic linkage studies of hypertension in Taiwan suggested some positive linkage signals in or near the *LPL* gene region with blood pressure (BP).[3 4] Because most Taiwanese have consanguinity with Chinese Han people, it is feasible and rational to verify these results in another homogeneous group.

Adducin is a membrane skeletal protein that is involved in the regulation of cellular signal transduction and membrane ion transport. Hypertension has also been linked to the α-adducin (*ADD1*) gene in some human studies.[5–7]

The role of the renin-angiotensin system in the pathogenesis of EH has been well documented; however, results of linkage of the angiotensin II receptor type 1 (*AT1*) gene with EH have been controversial among different populations.[8–10] Additionally, the relation between the vasopressin receptor 1A (*VAPR1A*) gene and EH is not established yet.[11]

In the current study, we used model free linkage analyses (SAGE/SIBPAL2 and variance component SOLAR) and the transmission/disequilibrium test (TDT/S-TDT) to examine linkage or potential linkage disequilibrium of genetic markers in the four candidate genes or their flanking genome regions to hypertension and BP in Chinese hypertensive families.

Key points

- Essential hypertension (EH) is a common, late onset disease that exhibits complex genetic heterogeneity, and is also the most common risk factor for cardiovascular, cerebrovascular, and renal diseases.
- The aim of this study was to examine whether seven microsatellite markers at four candidate genes (lipoprotein lipase (*LPL*) gene, α-adducin (*ADD1*) gene, angiotensin II receptor type 1 (*AT1*) gene, and vasopressin receptor 1A (*VAPR1A*) gene) or their flanking genome regions were linked or associated with EH in 148 Chinese hypertensive families.
- Using the linkage model in SOLAR, we identified a region of linkage with systolic blood pressure (SBP) to a 10.6 cM interval defined by markers D8S1145, D8S261, and D8S282 on chromosome 8, with a maximum two point lod score of 2.52 at the marker D8S261 and a maximum multipoint lod score (MLS) of 2.03 near the marker D8S261.
- Using SAGE/SIBPLA2 quantitative trait linkage analysis, there was a linkage of SBP and diastolic blood pressure (DBP) with the marker D8S261 (p=0.002 for SBP, and p=0.04 for DBP). In the qualitative trait linkage analysis, evidence for linkage between the marker D8S1145 and EH was found (p=0.029). TDT/S-TDT also supported significant linkage disequilibrium with EH at allele 3 of D8S261 (χ^2=8.643, p<0.01).
- These results indicated that the *LPL* gene and associated regions might contribute to individual BP variation in the Chinese population.

Table 1 Structure and number of sibs in families ascertained

Father	Mother	No of sibs per nuclear family 2	3	≥4	Total families
Affected	Affected	7	17	18	42
Affected	Non-affected	7	7	17	31
Non-affected	Affected	26	20	29	75

METHODS

Study population

All subjects were ethnic Han, who account for about 96% of the total population on the mainland of China. A total of 148 hypertensive families came from Beijing suburbs (Fangshan and Shijingshan districts), Jiangsu province (Changshu, Taixing, and Zhangjiagang districts), and Shanxi Province (Hanzhong city). Genotypes of pedigree members in 148 families were verified for Mendelian segregation. Table 1 lists the structure of sibs in families.

To be eligible for our study, one member of sib pairs in each nuclear hypertensive family had to meet the following criteria: (1) age older than 15 years; (2) either parent with hypertension; (3) two or more sibs with hypertension; (4) no clinical or biochemical indices of secondary hypertension: detailed clinical differentiation including clinical laboratory tests and ultrasound or computed tomography (CT) investigations on certain patients were implemented to exclude

Abbreviations: ADD1, α-adducin; AT1, angiotensin II receptor type 1; BP, blood pressure; CT, computed tomography; DBP, diastolic blood pressure; EH, essential hypertension; GRA, glucorticoid remediable aldosteronism; IBD, identity by descent; LPL, lipoprotein lipase; MLS, multipoint lod score; QTL, quantitative trait loci; SBP, systolic blood pressure; TDT/S-TDT, transmission/disequilibrium test

Table 2 Microsatellite markers for the candidate genes selected

Candidate gene	Chromosomal location	Marker	Heterozygosity	Genetic distance to gene (cM)
Lipoprotein lipase (*LPL*)	8p22	D8S1145	0.73	8
		D8S261	0.78	3.2
		D8S282	0.71	2.6
α-adducin (*ADD1*)	4p16.3	D4S2366	0.79	12.4
		D4S43	0.75	0.6
Angiotensin II receptor type 1 (*AT1*)	3q21-q25	CA repeat polymorphism	0.64	3′ flanking region
Vasopressin receptor 1A (*VAPR1A*)	12q14-q15	D12S398	0.67	7.3

patients with other known diseases, such as acute or chronic glomerulonephritis and pyelonephritis, glucorticoid remediable aldosteronism (GRA), Cushing's syndrome, or phaeochromocytoma; (5) not taking cortisone based or symphaticotonic drugs (oestroprogestogenic hormones by women) and absence of nephrolithiasis; and (6) resting-sitting SBP≥140 mm Hg or DBP≥90 mm Hg on three different occasions or use of antihypertensive drugs. Hypertensive families were initially screened by local doctors who had received rigorous training and passed the proficiency test. Once families were identified, epidemiologists and cardiologists from Fu Wai Hospital went to the local site to confirm the family eligibility. After positive confirmation, a formal study was conducted at an office in the local hospital. Trained interviewers using standard questionnaires collected information on demographic characteristics, history of cardiovascular disease, lifestyle factors, and family history of hypertension. Anthropometric measurements, including height, weight, and waist and hip circumferences, as well as an electrocardiogram were obtained by standard protocols. Measurements of BP were obtained by experienced doctors or nurses using a standard mercury sphygmomanometer with appropriately sized cuffs on the right arm after subjects had rested for 10 minutes. Three measurements were taken with an interval of at least 30 seconds between readings. The mean values of three consecutive measurements were used in these data. The study protocol was approved by the local research ethics committee of the Cardiovascular Institute and Fu Wai Hospital, Chinese Academy of Medical Sciences and Peking Union of Medical College. Informed consent was obtained from all study subjects.

Genotyping

Genomic DNA was obtained from peripheral blood leucocytes by phenol/chloroform extraction. Table 2 lists seven highly polymorphic microsatellite markers. The primer sequences were acquired from the genome database http://gdbwww.gdb.org/ and forward primer was labelled with fluorescence at the 5′ end.

Touch down polymerase chain reaction

Touch down polymerase chain reaction (PCR) was performed in thermocyclers of a Perkim Geneamp 9700 (Applied Biosystems, USA) in a 5 µl reaction volume. The amplifications involved initial denaturation for 10 minutes at 95°C, followed by 42 cycles each of denaturation for 30 seconds at 94°C, annealing for 40 seconds at 56°C, and extension for 60 seconds at 72°C, with the exception of the first 17 cycles, in which the annealing temperatures were decreased from 63°C to 56°C by 0.5°C per cycle. At the end of the amplifications, an extension reaction was performed at 72°C for 10 minutes.

The products from PCR were mixed with the DNA size standard (Genescan Rox-HD400, Applied Biosystems, USA), dextran blue dye (loading buffer), and formamide. The electrophoresis was carried out in 1 × TBE at 51°C, 3 KV for 2.5 hours using an ABI 377 sequencer (Applied Biosystems, USA). Genotyping was performed by Genescan 3.1 and Genotype 2.1 software (Applied Biosystems, USA). The entire allelotyping procedure was verified by genomic DNA from CEPH families 1347–02.

Statistical analysis

Values for SBP and DBP were standardised according to data from the third national BP sampling survey of China conducted in 1991, which involved about 940 000 subjects selected randomly and stratified according to the city and rural area from 30 provinces. Population mean SBP and DBP were 119.1 (SD 20.6) mm Hg and 73.0 (SD 11.6) mm Hg based on the data from that survey. Standardised BP values were obtained with the ratio of the difference between the subjects' BP values and the population mean BP values of 1991 to standard deviation. A transformation to the square root for SBP was done. A normal distribution test was conducted for SBP and DBP by SPSS 10.0.

The SAGE/SIBPAL2 4.0 program was used to perform qualitative trait and quantitative trait linkage analyses of D8S1145, D8S261, D8S282, D4S2366, D4S43, D12S398, and the CA repeat polymorphism in the 3′ flanking region of the *AT1* gene. This weakly parametric method, based on the Haseman-Elston algorithm, regressed the mean corrected cross product of sib pairs on the mean proportion of alleles' shared identity by descent (IBD).[12]

In addition, for the quantitative trait of BP, two point and multipoint linkage analyses were conducted by the variance component method in SOLAR 1.7.4.[13] The covariate effects evaluated were age, sex, and the product of age and sex.

Besides conventional TDT, an extended TDT (S-TDT and combined Z score) was also performed. Because EH is a disease with a late onset, sometimes the data from parents are difficult to obtain. In this case, a bias can arise in the TDT if the genotype for one parent is missing. Whereas S-TDT compares the marker genotypes in affected and unaffected offspring, there is no corresponding bias in the S-TDT. Therefore, a combined Z score was obtained by combining information from TDT and S-TDT and making Bonferroni's correction.[14]

RESULTS

Study sample

Table 3 shows the clinical characteristics of all subjects by parents and offspring.

The DBP followed a normal distribution (p=0.229) as did the SBP after a transformation to the square root (p=0.115).

SAGE/SIBPAL2 analysis

Qualitative trait linkage analysis showed that there was linkage evidence between the marker D8S1145 and EH (p=0.029). Some positive signals were seen only in chromosome 8 by quantitative trait linkage analysis (table 4). We obtained significant evidence for linkage of the D8S261 locus

Table 3 Clinical characteristics of 799 subjects

	Parents		Offspring	
	Affected	Unaffected	Affected	Unaffected
No	154	111	346	188
Male/female	57/97	75/36	225/121	109/79
Age (years)	72.14 (8.95)	71.73 (10.29)	47.89 (8.39)	43.13 (8.05)
BMI (kg/m^2)	24.29 (4.69)	22.46 (2.99)	26.25 (3.40)	24.72 (3.24)
SBP (mm Hg)	164.80 (23.93)	134.44 (26.43)	147.32 (20.09)	120.91 (16.18)
DBP (mm Hg)	87.61 (13.47)	75.97 (10.26)	94.80 (11.65)	79.16 (11.47)

Values are means (SD); BMI, body mass index;

Table 4 Quantitative trait linkage analysis of chromosome 8 for SBP and DBP

		SBP			DBP		
Marker	Sib pairs	β	SE	p value	β	SE	p value
D8S261	520	0.6688	0.2328	0.0021**	0.4792	0.2723	0.0395*
D8S282	423	0.0170	0.2365	0.4714	0.0471	0.2923	0.4360
D8S1145	477	0.2230	0.2072	0.1411	0.1988	0.2476	0.2112

*p<0.05; **p<0.01.

to SBP and DBP (p=0.002 and p=0.04, respectively). Allele sharing IBD analysis was performed separately for sib pairs with no, one, or two affected members. We detected a significant excess of two alleles sharing IBD above an expected sharing of 0.25 at the marker D8S261 (p=0.029 for affected and p=0.039 for unaffected sib pairs), as well as at the marker D8S1145 (p=0.013 for affected and p=0.038 for discordant sib pairs).

Two point and multipoint linkage analysis in SOLAR

Table 5 lists two point linkage lod scores of all markers selected and BP phenotypes. Two point linkage analysis for the *LPL* gene showed that there was evidence for linkage of SBP to the marker D8S261 (lod=2.52). However, there was no linkage (lod<1.0) for DBP and EH with either of those markers and no evidence of two point linkage was found in or near another three candidate genes (*ADD1*, *AT1,* and *VAPR1A*). With multipoint linkage analysis for the *LPL* gene, the markers D8S1145, D8S261, and D8S282 with SBP showed a suggestive linkage finding, with a maximum lod score of 2.03 near D8S261, but no such evidence of linkage of those three markers with DBP on chromosome 8 was identified, with a maximum lod score of 0.40. By means of a permutation, we obtained a significant adjusted p value from the marker D8S261 (p=0.0001) for SBP. However, no significant p values (p=0.5088 for DBP and p=0.5000 for EH) were found for DBP or EH or (p=0.5000) in or near the other three candidate genes (*ADD1*, *AT1,* and *VAPR1A*).

TDT/S-TDT analysis

A TDT/S-TDT analysis was performed after we had detected some positive linkage signals at markers D8S1145 and D8S261. We did not detect significant excess transmission of any allele for D8S1145 with EH. A significant association between D8S261 in the region of 8p22 and hypertension was found based on TDT/S-TDT analysis (table 6). Allele 3 of D8S261 showed significant linkage disequilibrium with EH in traditional TDT, S-TDT tests (χ^2=8.643, p<0.01; Z′=2.408, p<0.05) and performance after Bonferroni's correction (Z=3.517, p<0.01).

DISCUSSION

We have used Haseman-Elston and variance component based linkage methods to estimate the contribution of variation in the regions of the four candidate genes to interindividual variation in BP. Our results support the hypothesis that markers near the *LPL* gene are genetically linked to hypertension or BP in Chinese hypertensive families. Large samples of subjects residing in rural areas in China were used, where drugs for

Table 5 Blood pressure phenotypes and markers with two point lod scores and permutated p values

		Lod scores and permutated p value					
		SBP			DBP		
Candidate gene	Marker	a	b	p value	a	b	p value
LPL	D8S261	1.89	2.52	0.0001	0.41	0.24	0.5088
	D8S282	0	0	0.5097	0	0.02	0.5088
	D8S1145	0.87	0.52	0.1015	0.20	0.30	0.5088
ADD1	D4S2366	0.31	0.21	0.5000	0.02	0.01	0.5000
	D4S43	0	0	0.5000	0	0	0.5000
AT1	CA repeat polymorphism	0	0	0.5000	0	0	0.5000
VAPR1A	D12S398	0	0	0.5000	0	0	0.5000

a, lod scores before adjustment for age and sex; b, lod scores after adjustment for age and sex.

Table 6 TDT/S-TDT results for alleles of microsatellite D8S261

TDT					S-TDT					Combined scores	
Allele	Trans	Untrans	χ^2	p value	Y	Mean (A)	Var (V)	Z′	p value	Z′	p value
3	39	17	8.643	<0.01	204	186.8 48	47.814	2.408	<0.05	3.517	<0.01
Non-3	17	39	8.643	<0.01	84	71.429	20.601	2.660	<0.05	0.182	>0.05

Y, total observed numbers of the allele among affected sibs; A, mean of the numbers of the allele among affected sibs; V, variance of the allele among affected sibs; Z′, the correction of the Z score.
In combined scores, Z′ is Bonferroni's correction value.

treating hypertension are uncommon. These areas are appropriate for collecting hypertensive families for the study of quantitative traits in BP. This rural population is relatively homogeneous. The rate of awareness and treatment of hypertension was low. Most hypertensive patients did not take antihypertensive 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 examine the complete distribution of alleles underlying interindividual variation in the quantitative BP phenotype. Additionally, we had sufficient power to detect the previously found positive linkage signals in the Taiwanese sample if the *LPL* gene or markers near the genomic regions were indeed linked to hypertension in China. One genetic linkage study of young onset hypertension in Taiwan suggested a positive linkage between D8S1145 near the *LPL* gene and BP.[3] Another study of non-diabetic members of Taiwanese families also found significant linkage of an *LPL* gene marker (D8S261) with SBP.[4]

Our results are consistent with their finding. In our study, a linkage of the marker D8S261 with SBP was found, with a lod score of 2.52. Furthermore, multipoint linkage analysis disclosed a suggestive linkage with a maximum lod score of 2.03 with SBP near D8S261. Moreover, a linkage of the marker D8S261 with DBP was also found in our study. A possible difference between our study and the study by Wu *et al*[4] could be the phenotype used for the linkage analysis. It is also possible that a gene other than *LPL* in this region is responsible for linkage with DBP. Also, environmental variables often significantly affect phenotype and obscure genetic effects.

Although the way in which *LPL* is involved in the development of hypertension is not apparent, hypertension has been found to occur more often than expected in families with familial combined hyperlipidaemia. Membrane ion transport, which has been related to hypertension, can be altered by lipid abnormalities and could have some involvement in the mechanisms that link high triglyceride concentrations and hypertension.[3] A linkage analysis of EH with candidate genes involved in the regulation of glucose and lipid metabolism in 95 Chinese families by Chu *et al*[15] also provided a finding that the *LPL* gene could be linked to EH. However, a study by Hunt *et al*[16] involving a white population in the United States resulted in a negative finding.

The present study cannot clearly distinguish between contributions of the *LPL* gene itself and that of other genes near to D8S261, although the *LPL* gene is considered as a prime candidate gene responsible for BP variability in our study. For the *LPL* gene, the three markers selected span a region that contains this gene. In this region, defined by markers D8S1145, D8S261, and D8S282 on chromosome 8, other potential candidate genes are located, including the α-1C adrenergic receptor gene (*ADRA1C*) and a platelet derived growth factor receptor-like (*PDGFRL*) gene. The *ADRA1C* gene is a member of the adrenergic receptor superfamily, which influences heart rate, cardiac contraction, and vascular tone.[17] The α-1B adrenergic receptor gene (*ADRA1B*) was reported to show linkage with SBP.[18] Bray *et al*[19] reported a significant association between the polymorphism Arg16Gly within the β_2-adrenergic receptor gene (*ADRB2*) and SBP among discordant sibs. Another study reported a significantly higher frequency of the Gly16 allele at this locus in African-Caribbean hypertensive patients compared with normotensive controls.[20] The results from these linkage and association studies support the need for functional testing of 1B, β_2, and perhaps other adrenergic receptor genes, such as *ADRA1C*. It was postulated that the *ADRA1C* gene might be an important candidate in the regulation of peripheral blood flow and arterial pressure.

PDGFRL is a cytokine related receptor gene with a significant sequence similarity to the ligand binding domain of the platelet derived growth factor receptor β (*PDGFR*β) gene. A study showed that proliferation of mesangial cells requires a *PDGFR*β mediated signal transduction.[21] The impairment of kidney function relevant to proliferation of mesangial cells is involved in the generation of hypertension; therefore, *PDGFRL* may be a logical candidate gene contributing to the development of EH. Consequently, we cannot exclude the possibility that the marker D8S261 suggested linkage or association with other potential candidate genes in this region.

Recent studies have emphasised the importance of association studies as a means of localisation of genes for complex human disease, as the genetic distance over which useful linkage disequilibrium is thought to be present is much smaller than that over which linkage can be detected.[14] A TDT test that relies on linkage disequilibrium (the simultaneous presence of linkage and association) is often more powerful than other tests that rely on linkage alone for identification of markers closely linked to genes that contribute to disease susceptibility. Allele 3 of the marker D8S261 had a significant linkage disequilibrium with EH; the association with EH remained significant even after Bonferroni's correction. It was deduced that allele 3 carriers might have a higher incidence of risk of EH than allele 3 non-carriers. In addition, it was probable that the allele of D8S261 was in linkage disequilibrium with other closer genes than *LPL* in this region. We are looking forward to further studies to confirm this result.

The *ADD1* gene is regarded as a candidate for a "salt sensitivity gene". As envisioned by Cusi *et al*,[7] its variant has been shown to enhance Na^+-K^+ pump activity and increase renal tubular sodium reabsorption. However, the results of linkage or association studies on the *ADD1* gene are controversial among different populations.[6 22–24] Our results did not support linkage of the *ADD1* gene with EH. The *ADD1* polymorphism might account for only a portion of genetic variation of BP and be associated with a particular form of hypertension, characterised by alterations in sodium handling and response of plasma renin activity.

The renin-angiotensin system plays an important part in the function of the cardiovascular system and the regulation of BP. No linkage was shown between the CA repeat polymorphism in the 3′ flanking region of the *AT1* gene and interperson BP variation in our study. However, linkage of the CA repeat polymorphism with EH has been found in the Chinese Han, Tibetan, and Finnish populations.[8 10 25] In the Chinese

population, subjects had moderate or severe hypertension (SBP≥160 mm Hg or DBP≥95 mm Hg); therefore, we cannot exclude the possibility that the *AT1* gene may have an impact on interperson variation in BP, especially among those with clinically manifest hypertension.

Essential hypertension is considered to be a multifactorial disease resulting from a combination of several predisposing genes interacting with each other and environmental factors. Linkage studies of EH pose several problems, including delayed onset of phenotypic expression, varying penetrance, and lack of unequivocal diagnostic criteria.[26] When multiple genetic alleles contribute to a complex trait, linkage analysis has somewhat limited power for finding genes of modest effect. Also, environmental variables often significantly affect phenotype and obscure genetic effects. These factors may explain inconsistent linkage results.

In conclusion, we used two different weakly parametric linkage tests, sib pair linkage (SAGE/SIBPAL2) and pedigree based (SOLAR) approaches, to estimate the quantitative or qualitative contribution of variation at the four candidate genes or their near genomic regions to BP and EH. Positive linkage evidence of the *LPL* gene to hypertension and BP was found and TDT further supported this result. The *LPL* gene or other genes near to the D8S261 genomic region may influence individual BP variation in the Chinese population.

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Authors' affiliations

W J Yang, J F Huang, D L Ge, W Q Gan, G Y Huang, D F Gu, Division of Population Genetics, Cardiovascular Institute and Fu Wai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 167 Beilishi Road, Beijing 100037, China
W J Yang, D F Gu, Y Shen, B Q Qiang, National Human Genome Centre at Beijing, North Yongchang Rd 3–707, Beijing 100176, China
R T Hui, Sino-German Molecular Medicine Laboratory, Cardiovascular Institute, Fu Wai Hospital, Beijing, China
C L Yao, Nanjing Medical University School of Public Health, China
Z J Fan, Peking Union Hospital, Beijing, China

Correspondence to: Dr D F Gu, Division of Population Genetics, Cardiovascular Institute, Fu Wai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 167 Beilishi Road, Beijing 100037, China; gudf@yahoo.com

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