Evidence for linkage and association of the markers near the \textit{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

\textbf{E}ssential 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.\textsuperscript{1} Genetic linkage studies and genome wide scans have disclosed many possible candidate loci contributing to hypertension.\textsuperscript{2,3}

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 (\textit{LPL}) gene is a logical candidate gene that could contribute to the development of hypertension.\textsuperscript{4}

\textit{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 \textit{LPL} gene region with blood pressure (BP).\textsuperscript{5,6} 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 transport. Hypertension has also been linked to the \(\alpha\)-adducin (\textit{ADD1}) gene in some human studies.\textsuperscript{7,8}

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 (\textit{AT1}) gene with EH have been controversial among different populations.\textsuperscript{9,10} Additionally, the relation between the vasopressin receptor 1A (\textit{VAPR1A}) gene and EH is not established yet.\textsuperscript{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.

\textbf{METHODS}

\textbf{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 other causes.

<table>
<thead>
<tr>
<th>Key points</th>
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<td>\textbf{•} 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.</td>
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<td>\textbf{•} The aim of this study was to examine whether seven microsatellite markers at four candidate genes (lipoprotein lipase (\textit{LPL}) gene, (\alpha)-adducin (\textit{ADD1}) gene, angiotensin II receptor type 1 (\textit{AT1}) gene, and vasopressin receptor 1A (\textit{VAPR1A}) gene) or their flanking genome regions were linked or associated with EH in 148 Chinese hypertensive families.</td>
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<td>\textbf{•} 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.</td>
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<td>\textbf{•} Using SAGE/SIBPAL2 quantitative trait linkage analyses, 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 ((\chi^2=8.643, p&lt;0.01)).</td>
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<td>\textbf{•} These results indicated that the \textit{LPL} gene and associated regions might contribute to individual BP variation in the Chinese population.</td>
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\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Father} & \textbf{Mother} & \textbf{No of sibs per nuclear family} & \textbf{Total families} \\
\hline
\textbf{Affected} & \textbf{Affected} & 7 & 17 & 18 & 42 \\
\textbf{Affected} & \textbf{Non-affected} & 17 & 7 & 17 & 31 \\
\textbf{Non-affected} & \textbf{Affected} & 26 & 20 & 29 & 75 \\
\hline
\end{tabular}
\caption{Structure and number of sibs in families ascertained.}
\end{table}

Abbreviations: ADD1, \(\alpha\)-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.

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patients with other known diseases, such as acute or chronic glomerulonephritis and pyelonephritis, glucocorticoid remediable aldosteronism (GRA), Cushing’s syndrome, or phaeochromocytoma; (5) not taking cortisone based or sympathico-adrenal aldosteronism (GRA), Cushing’s syndrome, or phaeochromocytoma.

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, and the association of the marker D8S1145 and the CA repeat polymorphism with hypertension. The SAGE/SIBPAL2 4.0 program was used to perform qualitative and quantitative trait linkage analyses of D8S1145, D8S261, D8S282, D4S2366, D4S43, D12S398, and the CA repeat polymorphism, and the association of the marker D8S1145 and the CA repeat polymorphism with hypertension. The SAGE/SIBPAL2 4.0 program was used to perform qualitative and quantitative trait linkage analyses of D8S1145, D8S261, D8S282, D4S2366, D4S43, D12S398, and the CA repeat polymorphism, and the association of the marker D8S1145 and the CA repeat polymorphism with hypertension.
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 ($\chi^2=8.643$, 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...
treatment of hypertension is not apparent, hypertension has been suggested 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.16 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., 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.17-20 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 inter-personal BP influences 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

<table>
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<tr>
<th>Allele</th>
<th>Trans</th>
<th>Untrans</th>
<th>( \chi^2 )</th>
<th>p value</th>
<th>Y</th>
<th>Mean (A)</th>
<th>Var (V)</th>
<th>Z</th>
<th>p value</th>
<th>Z'</th>
<th>p value</th>
</tr>
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<tr>
<td>3</td>
<td>39</td>
<td>17</td>
<td>8.643</td>
<td>&lt;0.01</td>
<td>204</td>
<td>186.848</td>
<td>47.814</td>
<td>2.408</td>
<td>&lt;0.05</td>
<td>3.517</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Non3</td>
<td>17</td>
<td>39</td>
<td>8.643</td>
<td>&lt;0.01</td>
<td>84</td>
<td>71.429</td>
<td>20.601</td>
<td>2.660</td>
<td>&lt;0.05</td>
<td>0.182</td>
<td>&gt;0.05</td>
</tr>
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</table>

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.

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

reported a significant association between the polymorphism Arg16Gly within the \( \beta \)-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.26 The results from these linkage and association studies support the need for functional testing of \( \beta \), \( \beta \), and perhaps other adrenergic receptor genes, such as ADRAIC. It was postulated that the ADRAIC gene might be an important candidate in the regulation of peripheral blood flow and arterial pressure. PDGFR\( \beta \) is a cytokine related receptor gene with a significant sequence similarity to the ligand binding domain of the platelet derived growth factor receptor \( \beta \) (PDGFR\( \beta \)) gene. A study showed that proliferation of mesangial cells requires a PDGFR\( \beta \) mediated signal transduction.27 The impairment of kidney function relevant to proliferation of mesangial cells is involved in the generation of hypertension; therefore, PDGFR\( \beta \) 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.

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 \( \alpha \)-1C adrenergic receptor gene (ADRA1C) and a platelet derived growth factor receptor-like (PDGFR\( \alpha \)) gene. The ADRA1C gene is a member of the adrenergic receptor superfamily, which influences heart rate, vascular tone, and vascular tone.22 The \( \alpha \)-1B adrenergic receptor gene (ADRA1B) was reported to show linkage with SBP24 Bray et al.24 reported a significant association between the polymorphism Arg16Gly within the \( \beta \)-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. The results from these linkage and association studies support the need for functional testing of \( \beta \), \( \beta \), and perhaps other adrenergic receptor genes, such as ADRAIC. It was postulated that the ADRAIC gene might be an important candidate in the regulation of peripheral blood flow and arterial pressure.

PDGFR\( \beta \) is a cytokine related receptor gene with a significant sequence similarity to the ligand binding domain of the platelet derived growth factor receptor \( \beta \) (PDGFR\( \beta \)) gene. A study showed that proliferation of mesangial cells requires a PDGFR\( \beta \) mediated signal transduction. The impairment of kidney function relevant to proliferation of mesangial cells is involved in the generation of hypertension; therefore, PDGFR\( \beta \) 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.

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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 inter-personal 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. 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 DBS261 genomic region may influence individual BP variation in the Chinese population.

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