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Genome-Wide Scan for Blood Pressure in Australian and Dutch Subjects Suggests Linkage at 5P, 14Q, and 17P

Jouke-Jan Hottenga, John B. Whitfield, Danielle Posthuma, Gonneke Willemsen, Eco J.C. de Geus, Nicholas G. Martin, Dorret I. Boomsma

Abstract—Large-scale studies estimate the heritability of blood pressure at $\approx 50\%$. We carried out a genome-wide linkage analysis to search for chromosomal loci that might explain this heritability using longitudinal, multiple measures of systolic and diastolic blood pressure obtained in sibling pairs and dizygotic twin pairs from 2 countries (a total of 286 pairs from Australia and 636 pairs from the Netherlands). These pairs and a large number of their parents were genotyped with microsatellite markers. Multivariate linkage analysis of the combined data of both countries, using a variance components approach, showed suggestive linkage for diastolic blood pressure on chromosomes 5p13.1 (logarithm of odds score: 2.48), 14q12 (logarithm of odds score: 2.40) and 17q24.3 (logarithm of odds score: 2.36). The highest logarithm of odds score of 1.21 for systolic blood pressure was observed on chromosome 13q34. These results replicate earlier findings and add to a slowly emerging picture of multiple loci contributing to quantitative blood pressure variation. (*Hypertension*. 2007;49:832-838.)

Key Words: epidemiology ■ twin study ■ blood pressure ■ genetic linkage ■ longitudinal studies ■ candidate genes

Hypertension is a common and complex disorder that affects 31.0% of people ≤ 45 years of age and 77.6% of subjects ≤ 75 years,¹ thereby substantially increasing their risk for cardiovascular disorders, notably ischemic heart failure and stroke.² Numerous studies have shown that genetic factors play a role in hypertension, as well as in the underlying quantitative trait, blood pressure (BP).³ The heritability for both systolic BP (SBP) and diastolic BP (DBP) is $\approx 50\%$ and is remarkably stable over populations and time, but the identification of quantitative trait loci (QTL) influencing BP and hypertension is a complex endeavor.⁴⁻⁸ Many genome scans have been performed, but most of the reported BP loci are not yet robustly replicated, and larger studies and meta-analyses of data tend to show less significant results.^{7,8} Reasons for these findings may include a large number of genes with small effects involved, which are difficult to detect; true heterogeneity between populations; the sometimes relatively small sample sizes; different designs and analytical approaches; different definitions of the phenotype (hypertension or quantitative BP scores); different treatment of subjects who take antihypertensive medication; and the use of different covariates.

In 2 recent studies from the Netherlands Twin Register and the Australian Twin Register, we found that the genetic variance in longitudinal BP data is largely attributable to stable genetic factors.^{6,9} Simulations have shown that the use of such longitudinal data sets increases the power to detect

QTLs.¹⁰ We carried out a scan on repeated measures of BP over a 7- to 12-year period to localize genes that may explain BP heritability.

Methods

Subjects

Longitudinal BP data for 3472 monozygotic (MZ) twins, dizygotic (DZ) twins, and siblings were collected from 7 studies that were performed in the Australian Twin Register (n=3) and Netherlands Twin Register (n=4).¹¹⁻¹⁹ BP data from all of the possible quasi-independent sibling pairs with ≥ 1 measure in both siblings were used for linkage analysis (MZ twin pairs with an additional sibling counted once). The average time between the longitudinal measures was 12 years in Australia and 7 years in the Netherlands. Subjects were recruited on a voluntary basis from the general population.¹¹⁻¹⁹ Recruitment of subjects for the first Australian study was based on word of mouth, media appeals, and advertising.¹⁵ The second and third studies were a follow-up of this sample, where in the third study, subjects were added who joined the Australian Twin Register during various (telephone) surveys of other studies.^{16,17} For the Netherlands, recruitment for the first study was through city council population registries. Subjects for the other studies were recruited through city council population registries, advertisements in twin newsletters, and other media.^{11,14} In consecutive Netherlands Twin Register studies, earlier participating twins might participate, and new subjects were added. The studies were approved by the appropriate ethics committees, and informed consent was obtained from all of the participants.

Measures

BP during rest was obtained using slightly different methods and included manual readings by a nurse,¹⁵ brachial cuff measurements

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with an automated Dynamap 845 recorder,^{11,12,14,16,17} and assessment with an ambulatory monitor (Spacelabs 90207).¹³ A mean of 2 to 6 BP measures was taken as the resting value. In the Dutch ambulatory BP study, the mean (3.8) of all evening measures when subjects were seated quietly was used. If subjects took antihypertensive medication, a correction of +14 mm Hg for SBP and +10 mm Hg for DBP was made for the nonambulatory studies.^{6,20,21} For the ambulatory study, the correction was drug class specific, and mean values were close to +14 mm Hg for SBP and +10 mm Hg for DBP.¹³

Genotyping

DNA was extracted from either whole blood or buccal swabs using standard protocols.^{22,23} Samples were genotyped by the Mammalian Genotyping Service in Marshfield, the Molecular Epidemiology Section, Leiden University Medical Centre, Sequana, or Gemini (Australian samples).²⁴ The genotype data from these screens were combined but were kept separate for each country. Pedigree relations were checked with Graphic Representation of Relationships.²⁵ Errors of Mendelian inheritance were detected with Pedstats.²⁶ Markers and samples were removed if their total error rate was >1%; in all of the other cases, the specific erroneous genotypes were set as unknown. Unlikely recombinants were detected using Merlin, and erroneous genotypes were removed with Pedwipe.²⁶ Sibling pairs were selected that had ≥ 200 autosomal markers genotyped for each individual. This resulted in a sample of 286 pairs for Australia. On average, 807 markers were tested per sibling (206 to 1648) with an average heterozygosity of 76% and an average centimorgan spacing of 5.6. For the Netherlands, 636 pairs were available. In the Dutch families, 544 parents were genotyped. Their information was used to obtain estimates of identity-by-descent (IBD) status in the offspring. The average number of markers genotyped in siblings was 383 (range: 201 to 743), and the average heterozygosity of autosomal markers was 76% with an average spacing of 9.7 cM. For the statistical analyses, the Haldane mapping function was used; all of the reported values are in Kosambi centimorgans. Marker positions were interpolated via locally weighted linear regression from National Center for Biotechnology Information build 35.1 physical map positions and the Rutgers genetic map.^{27,28} Data from 648 MZ pairs were included to estimate the contribution of background heritability.

Statistical Analysis

We estimated the probabilities that 0, 1, or 2 alleles are shared IBD for each sibling pair using a 2 cM spacing multipoint scan with Merlin.²⁶ When parents were genotyped, their information was used. When parents were not genotyped, IBD was estimated using population marker allele frequencies that were obtained from the observed genotype data from each country.

Multivariate linkage analysis was carried out using structural equation modeling as implemented in the program MX using the data of all of the possible sibling pairs.²⁹ The multivariate structure consisted of the 3 or 4 longitudinal measurements in Australian and Dutch studies, respectively. Data from the 2 countries were first analyzed separately and next combined. The total variance of BP was partitioned into the sources of variation because of additive genetic components (A), nonshared environmental influences (E), and the variance explained by a putative QTL (Q). The QTL was modeled as a single latent factor that explained an equal amount of variance in all of the measurements, that is, the factor loadings were constrained to be equal over time. Modeling the QTL in such a way focuses the linkage analysis on genes that influence BP across age. The localization of age-specific QTLs was not modeled, because our previous studies showed that such QTLs did not appear to be present.^{6,9}

To accommodate any residual unique environmental and genetic (co)variance, A and E were modeled using a full triangular decomposition.^{6,9} For DZ twin and sibling pairs, the covariance between sibling pairs was modeled as: $0.5A + \hat{\pi}_{ijk}Q$, where $\hat{\pi}_{ijk}$ equals the proportion of alleles shared IBD between siblings j and k for the i -th family. For MZ twins, the covariance was modeled as $A + Q$, because they share their full genome. The model for the means included a linear regression of age and sex for each study (allowing for study heterogeneity between measurements) and was modeled

simultaneously with the ANOVA components. Significance of the genetic variation because of a QTL was evaluated by likelihood ratio tests comparing the model with the Q variance component to the model without. The resulting χ^2 difference was divided by $2\ln 10$ ($=4.6$) to obtain the logarithm of odds (LOD) score. Nominal 1-sided P values were calculated using a 50:50 $\chi^2_{0.1}$ distribution, a 50:50 mixture of a χ^2_0 distribution with a point mass of 0, and a χ^2_1 distribution. Accordingly, a significance level of α was obtained from a χ^2_1 critical value of 2α .

In the joint analysis, heterogeneity and homogeneity of the QTL effect between the 2 countries was considered. Under heterogeneity, all of the model parameters (A , E , and Q) between countries were freely estimated. Using a likelihood-ratio test (χ^2_1), we first tested the assumption of homogeneity in the variance associated to a putative QTL by restricting Q to be the same across both countries while allowing other parameters to differ.³⁰ If homogeneity of Q is observed ($P \geq 0.05$), a combined linkage analysis can be used next to determine the significance of the QTL effect on the basis of the pooled data set using the $\chi^2_{0.1}$ distribution without an increased threshold for significance (because there is no extra free parameter because of heterogeneity). Therefore, we report replicated loci with LOD scores > 1.18 ($P = 0.01$), as well as new loci with an LOD > 2.2 ($P = 0.00074$), the asymptotic threshold for suggestive linkage as suggested by Lander and Kruglyak.³¹ The 1-LOD drop support interval was used as an estimate for the 90% CI of any QTL location³² and was used to examine linkage replication compared with previously reported locations and candidate genes (using Ensembl: <http://www.Ensembl.org> and OMIM: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>).

Results

The number of individuals, number of subjects using antihypertensive medication, male–female ratio, average age, and average BP levels are shown in Table 1. No differences in BP levels were found between MZ and DZ twins and siblings. In the later studies, an increase in age range and BP variance is seen (study 3 from Australia and studies 3 and 4 from the Netherlands). Between the 2 countries, the age range is comparable. Age and sex differences were described previously in detail.^{6,9} Briefly, SBP levels in men were ≈ 5 mm Hg higher than in women. DBP levels in men were generally ≈ 3 mm Hg higher than in women. Increasing BP with increasing age was seen in all of the studies, but the age effect did not always reach significance ($P > 0.01$). There were no sex differences in MZ correlations or DZ same-sex versus opposite-sex correlations ($P > 0.01$), suggesting that the same genes influence BP in men and women.

In Figure 1, the linkage results of the country-specific genome scans are presented for SBP. The best LOD scores of 1.67 ($P = 0.0028$) and 1.27 ($P = 0.0078$) for SBP were found at chromosome locations 5q35.1 and 11q24.2 in Australia. For the Netherlands, the highest LOD score, 1.21 ($P = 0.0092$), was found on chromosome 13q34 for SBP. Figure 2 shows the linkage analyses for DBP. In the Netherlands Twin Register sample, suggestive linkage was obtained for chromosome 5p13.1 with an LOD score of 2.50 ($P = 0.00034$). In the Australian Twin Register, this location was not replicated, however. At a lower pointwise significance level for replication (LOD > 1.18 ; $P < 0.01$), both SBP and DBP showed a signal at 5q35.1 and 11q24.2. Locations showing increased IBD sharing for either DBP or SBP were at 3p14.1, 5p13.1, 5q35.1, 7p21.3, 7q21.13, 9q33.1 to 34.2, 10p12.31, 11q13.4 to 11q24.2, 13q34, 14q12, 17p12, 17q24.3, and 19p13.3.

TABLE 1. Characteristics of Studied Individuals With Genotype and Phenotype Data

Country	Group	Variable	Study 1	Study 2	Study 3	Study 4*
Australia	MZ	N	176 (2)	110 (1)	638 (60)	
		Male/female	86/90	48/62	188/450	
		Age	24.3 (5.3)	34.2 (5.0)	45.7 (11.9)	
		SBP	114.4 (13.8)	119.3 (14.4)	128.7 (17.7)	
		DBP	71.0 (11.8)	73.3 (11.0)	77.9 (11.9)	
	DZ	N	212 (3)	142 (1)	455 (38)	
		Male/female	93/119	61/81	170/285	
		Age	22.3 (4.3)	33.6 (4.6)	43.0 (11.0)	
		SBP	113.4 (13.6)	120.1 (11.8)	127.3 (16.8)	
		DBP	71.0 (11.0)	73.6 (9.2)	77.6 (11.5)	
Netherlands	MZ	N	140 (0)	190 (10)	261 (10)	148 (7)
		Male/female	70/70	92/98	117/144	53/95
		Age	16.3 (2.0)	44.2 (6.7)	38.1 (12.8)	30.6 (11.5)
		SBP	117.4 (7.5)	125.1 (12.9)	125.8 (14.8)	126.4 (11.4)
		DBP	66.6 (5.7)	77.2 (10.6)	76.1 (11.3)	77.6 (9.2)
	DZ and siblings	N	164 (0)	212 (16)	313 (21)	364 (11)
		Male/female	80/84	102/110	130/183	146/218
		Age	17.1 (2.0)	44.1 (6.6)	36.8 (12.9)	32.9 (11.8)
		SBP	117.5 (8.0)	126.3 (14.2)	126.6 (15.1)	126.1 (11.6)
		DBP	66.9 (6.7)	77.0 (10.3)	77.3 (10.9)	77.3 (9.1)

N indicates the total number of individuals in each study, in brackets the number of persons using medication. Age indicates age at time of measurement.

*This study measured BP with an ambulatory device during quiet sitting in the evening.

Table 2 summarizes the results of the joint genome scan. By combining the data of the 2 countries in a single scan, 3 locations with suggestive linkage were identified for DBP, which would not have been detected using the data in the individual countries alone. No significant heterogeneity was found for these locations, with P values of 0.56, 0.93, and 0.47, respectively. The 3 loci were 5p13.1 at marker ATAG022, 14q12 at marker GATA43H01, and 17p12 at marker D17S921, with maximum LOD scores of 2.48 ($P=0.00037$), 2.40 ($P=0.00045$), and 2.36 ($P=0.00049$), respectively. For SBP, no locations with suggestive linkage

were identified. Locations for the joint scan with nominal $P_s < 0.01$ for either SBP or DBP, which were identified previously in other studies, are also shown in Table 2. For the locations on chromosomes 5p13.1, 14q12, and 17p12, more details are depicted in Figure 3.

Discussion

This study presents suggestive linkage for DBP on 3 locations, namely, 5p13.1, 14q12, and 17p12. For SBP, no suggestive evidence for linkage was found. Several locations, found previously in >1 study, have been replicated to some

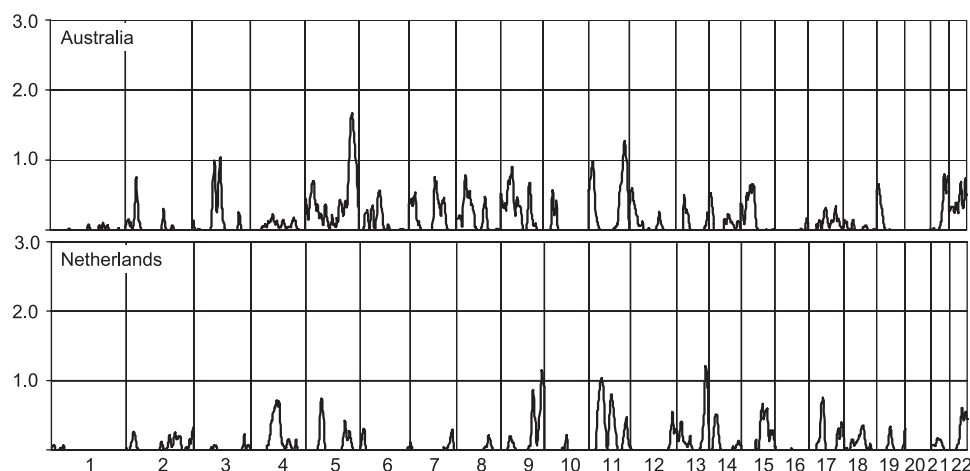


Figure 1. Multipoint linkage results for SBP in Australia and the Netherlands. On the horizontal axis, the chromosome number is indicated. The vertical axis indicates the LOD score.

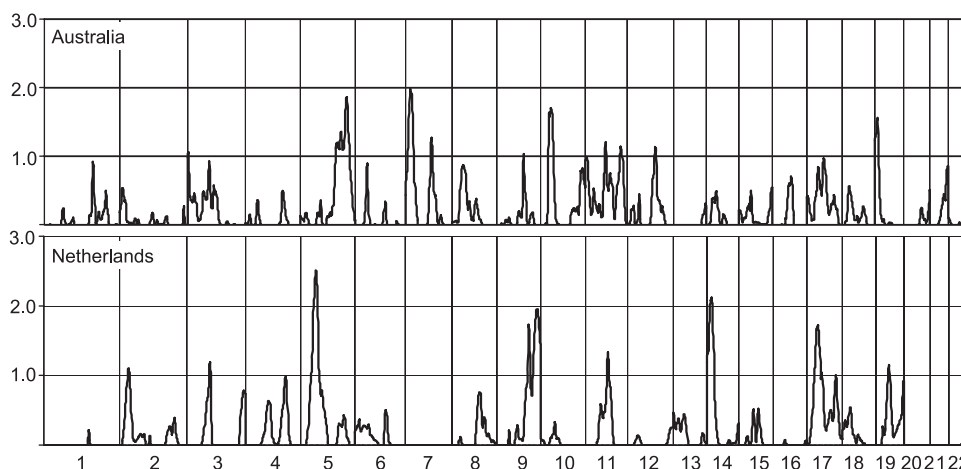


Figure 2. Multipoint linkage results for DBP in Australia and the Netherlands. On the horizontal axis, the chromosome number is indicated. The vertical axis indicates the LOD score.

extent (LOD: >1.18; $P < 0.01$) in our study, namely, 3p14.1, 5p13.1, 5q35.1, 7p21.3, 7q21.13, 9q33.1 to 34.2, 10p12.31, 11q13.4 to 11q24.2, 13q34, 14q12, 17p12, 17q24.3, and 19p13.3.^{8,33–40} However, the significance of the loci in the individual studies is often low, the regions are broad, and the significance tends to go down when the sample size is increased.^{7,8} This pattern of findings can be caused by several factors, but it is likely that many genes contribute to BP variance and that each has only a small effect. With current study sample sizes, locations of these genes may be missed.^{31,40} To increase power, several approaches can be used, such as pooling across samples and the use of repeated measures in a longitudinal design, as was done in the present study.

Medication use may affect QTL detection, but is unlikely to have been very important here. The average age at time of participation in most studies is still relatively low for frequent use of antihypertensive medication. Roughly 4% of the subjects were using antihypertensive medication. These subjects were not excluded, because previous studies have shown that excluding medicated hypertensive persons from the analysis reduces the genetic effect and power to detect

QTLs.^{20,21,41} Instead, we added the published treatment effects of their medication to the observed BP. When we examined which sibling pairs were responsible for the increased LOD scores at putative locations, a general finding was that concordant low BP pairs contributed most and not the pairs with high BP (data not shown). Note that this finding may be important for replication in studies examining only hypertensive pairs. Also, it indicates that there was probably a small influence of genes related to secondary forms of hypertension (caused by, eg, renal disorders or type 2 diabetes), which has not been excluded in this study.

Many candidate genes are located within the 90% CI of the 3 suggestive loci at 5p13.1, 14q12, and 17p. For the 5p13.1 location, a candidate gene is the atrial natriuretic peptide clearance receptor precursor gene (*NPR3*). The natriuretic peptides elicit a number of vascular, renal, and endocrine effects that are important in the maintenance of BP.⁴² The *NPR3* gene has already been associated with familial hypertension, but confirmation is still needed.⁴³ Two other candidates in the 5p13.1 region are the insulin gene enhancer protein gene (*ISLI*) and the lipid phosphate phosphohydro-

TABLE 2. Genome-Wide Scan Locations With $P_s < 0.01$ from the Joint Linkage Analysis of the Australian and Netherlands Longitudinal Blood Pressure Studies

BP	Chromosome	Position, cM	Marker	No Heterogeneity	Homogeneity Model	
				<i>P</i>	LOD	<i>P</i>
DBP	3p14.1	88.2	D3S1285	0.40	1.60	0.0033
DBP	5p13.1	60.8	ATAG022	0.56	2.48	0.00037
DBP	9q33.1	121.6	D9S1776	0.36	1.54	0.0039
DBP	9q34.2	154.9	CTAT016	0.20	1.59	0.0034
DBP	11q13.4	84.3	D11S2371	0.41	1.73	0.0024
SBP	13q34	113.7	D13S1315	0.67	1.21	0.0091
DBP	14q12	19.6	GATA43H01	0.93	2.40	0.00045
DBP	17p12	43.1	D17S921	0.47	2.36	0.00049
DBP	17q24.3	109.8	GATA63G01	0.66	1.24	0.0083

Heterogeneity testing: *P* value for the test of the assumption that there is no heterogeneity of the QTL effect between the 2 countries. Linkage under the homogeneity model: QTL analysis under the assumption that an equal amount of variance is explained by the QTL in the 2 countries. Positions in Kosambi centimorgans.²⁷

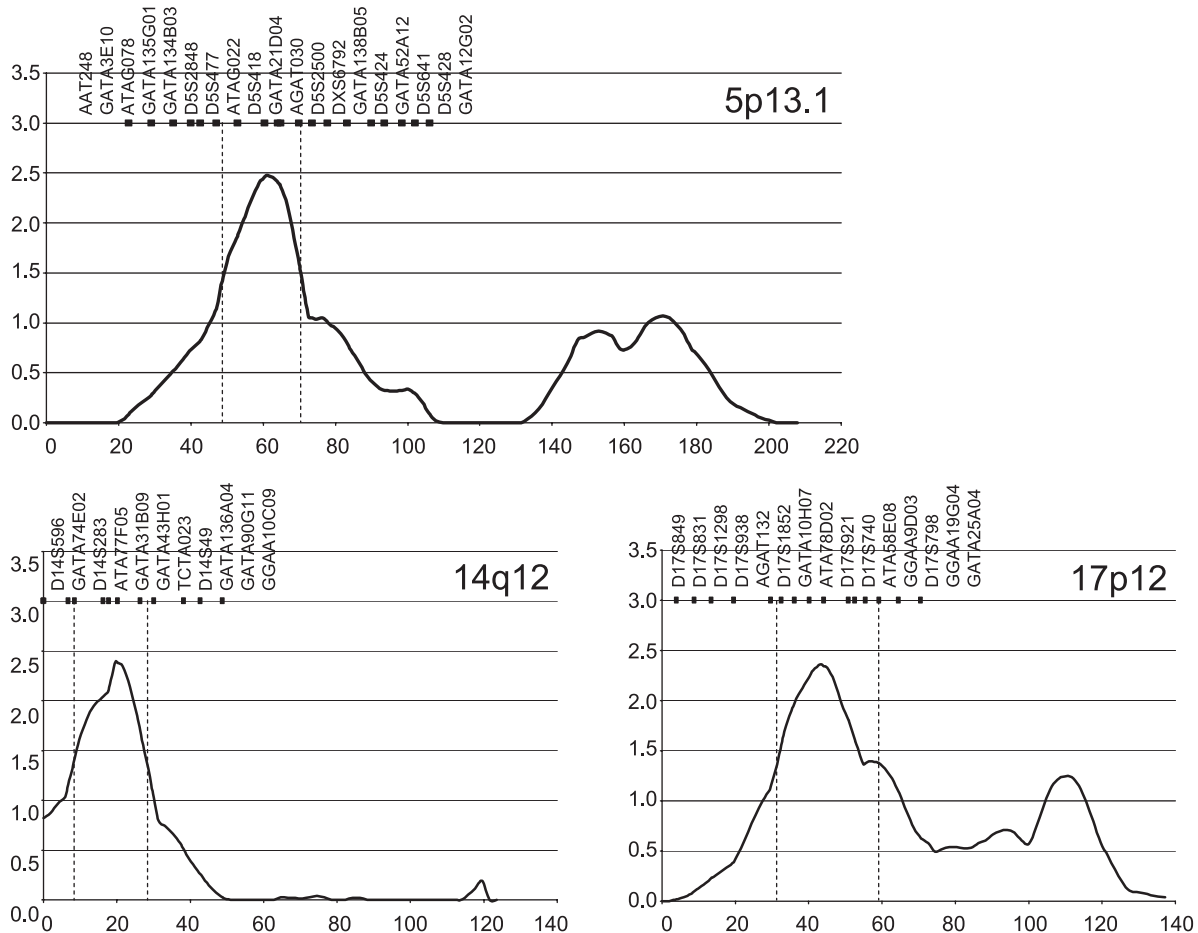


Figure 3. Detailed maps of the chromosomes with suggestive linkage for DBP. On the horizontal axis, the position on the chromosome is indicated in Kosambi centimorgans. On the vertical axis, the LOD score is given. The vertical dotted lines indicate the 90% CI around the maximum LOD score. On top, the tested markers are presented and in the right corner the chromosome band.

lase 1 gene (*PPAP2A*). Insulin metabolism, lipid metabolism, and BP regulation are strongly intertwined, and detrimental shifts in these risk factors for cardiovascular disorder often co-occur (the metabolic syndrome), possibly because of a joint genetic underpinning.^{44–46}

For the second region on chromosome 14q12, 2 candidate genes were found, namely the low-density lipoprotein receptor-related protein 10 precursor gene (*LRP10*) and the chymase precursor gene (*CMA1*). The second is related to the angiotensin pathway and causes hypertension and arteriopathy in rats when the gene is upregulated.⁴⁷ However, in a large study with Japanese subjects, variations in this gene did not show any association with BP.⁴⁸

For the third region, 17p12, the 90% CI is substantial. Several studies have indicated that there is probably >1 gene regulating BP on chromosome 17.⁴⁹ Because BP depends on salt balance, allelic variants of genes that influence this balance can be considered as potential candidates. In the 17p12 region, there are several of those genes, namely the NO synthase IIB and IIC genes (*NOS2A* and *NOS2B*), the solute carrier family 13-member 2 renal sodium/dicarboxylate cotransporter gene (*SLC13A12*), the solute carrier family 5 sodium/glucose cotransporter member 10 gene (*SLC5A10*), and the ATP-sensitive inward rectifier potassium channel 12

gene (*KCNJ12*).^{49–51} Other candidates are genes involved in syndromes caused by more severe DNA alterations; 2 located near 17p12 are neurofibromatosis (*NFI* gene) causing renal vascular damage^{52,53} and platelet glycoprotein IV deficiency (*CD36* gene) leading to a changes in all of the factors involved in the metabolic syndrome.^{54,55} Another candidate involved in lipid metabolism within the 17p12 region is the fatty aldehyde dehydrogenase gene (*ALDH3A2*). Furthermore, at each candidate location, genes related to the ubiquitin–protein ligase family were present. Ubiquitin has been linked to angiotensin II pathways and Na⁺ channel function in endothelial cells.^{56–58} These genes could, therefore, be considered potential candidates as well.

Perspectives

In this study, we found suggestive linkage for DBP at 5p13.1, 14q12, and 17p12. These loci have been replicated, and our study indicates further evidence for their involvement. Fine mapping of these regions, preferably by dense coverage of single nucleotide polymorphism markers, followed by family based association studies, is now a first priority. Next, functional variants that predispose to hypertension or a lower BP instead can be identified, as well as the pathways involved.

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Disclosures

None.

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