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Gene by Smoking Interaction in Hypertension: Identification of a Major QTL on Chromosome 15q for Systolic Blood Pressure in Mexican Americans

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Abstract

OBJECTIVE—Our objective was to investigate the influence of gene by smoking (GxS) interaction on hypertension (HT) and blood pressure (BP) using genome-wide linkage analysis in Mexican Americans, followed by SNP fine mapping of candidate genes in the linked chromosomal region.

METHODS—We used nonparametric methods to test for linkage of microsatellites with HT and BP measures in smokers, non-smokers, and the combined group. To begin fine-mapping of a major QTL for SBP on chromosome 15q that showed strong evidence for GxS interaction, we genotyped 55 SNPs in 9 candidate genes for association studies using two population-based statistical methods.

RESULTS—The strongest evidence for GxS interaction (p = 0.0004) was found for SBP on chromosome 15q, where a major QTL (LOD = 3.36) was identified only in non-smokers. Follow-up studies identified three SNPs in three genes (ANPEP, IGF1R, and SLCO3A1) that showed associations with SBP only in non-smokers, cumulatively accounting for a 7 mmHg increase in SBP. However, conditional linkage analyses that accounted for phenotypic effects of these SNPs only slightly reduced the original LOD score.

CONCLUSION—The detection of a major QTL on chromosome 15q for SBP in non-smokers indicates the presence of loci that influence BP via GxS interactions. However, identification of the genes that underlie such QTL effects remains a challenge. Although we found three candidate genes that showed significant associations with SBP in non-smokers, further studies are required to identify the gene(s) that underlie the chromosome 15q QTL that influences SBP via GxS interactions.

Keywords

genome wide scan; gene by environment interaction; hypertension; blood pressure; smoking; linkage analysis; association analysis; fine mapping

INTRODUCTION

Hypertension is a complex disease that results from interactions among many environmental factors (e.g. stress, physical inactivity, obesity, diet, smoking) and genetic factors. Many previous genome linkage scans and meta-analyses have searched for quantitative trait loci (QTLs) for blood pressure (BP) and hypertension (HT) [1–8]. These genome scans produced

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a long list of QTLs on every chromosome in the human genome, but few have been replicated and causative genes have not been identified. A major reason for inconsistency between studies may be different environmental modifiers that interact with genes to influence BP and HT. Although the deleterious effects of smoking on the cardiovascular system are well established, the relationship of smoking to BP is not yet settled [9]. Some studies have reported that smoking is associated with increased BP [10–14], others reported associations with decreased BP[15–17], while some found no effect at all [18,19]. However, smoking status has proven to be an important mediator of genetic effects in association studies of BP and HT [20–24].

The role of smoking in HT and BP regulation is not clear, but several mechanisms have been suggested to explain this relationship. Nicotine has a powerful stimulation effect on the sympathetic nervous system by increasing the production of neurotransmitters such as dopamine and nitric oxide (NO). These neurotransmitters can increase BP through catecholamine-mediated actions, or through their deleterious effects on the blood vessels [25,26]. Cigarette smoking also may raise BP by enhancing the release of endothelin-1, which is a very powerful systemic vasoconstrictor compound [10].

Mexican Americans (MA) are the fastest growing minority population in the US, but are relatively understudied in genome linkage scans for HT and related traits. Previous studies of MA families from San Antonio reported significant evidence of linkage for DBP on chromosome 2p11[27] as well as for longitudinal changes in SBP and mean arterial BP on chromosome 11q24.1 [28]. Significant evidence of linkage was also found for blood pressure factor (BPF) [29] and pulse pressure (PP) [30] on 17q23.1 in MA families from Starr County, Texas. In Hispanic families from California, significant evidence of linkage was reported for SBP and insulin resistance on chromosome 7q [31].

To our knowledge, smoking status has not been considered as a modifier in linkage genome scans for hypertension and related traits. Here we report the results of genome scans for HT and BP measures in MA families from Starr County using linkage analyses within separate groups of smokers and non-smokers, as well as the combined group. In addition, we report the results of follow-up association analysis for candidate genes located in a region of chromosome 15q that showed linkage for SBP only in non-smokers, as well as strong evidence for GxS interaction.

MATERIALS AND METHODS

Subjects and Measurements

The study sample consisted of MA families from Starr County, Texas, that participated in the Genetic Epidemiology Network of Arteriopathy (GENOA) study. MA subjects (n=1649) recruited for this study were members of families with at least two siblings with type II diabetes (total 420 families). SBP and DBP were measured on the dominant arm with the proper cuff size using the Dinamap oscillometric device. Three measurements per subject were taken in the sitting position, after 5 min rest before the first measurement and 1 min lapse between two readings, and the average of the three measurements was used for analysis. Hypertensive status was based on SBP≥140 mmHg or DBP≥90 mmHg, or taking antihypertensive medication. Smokers (current and previous) and non-smokers (never) were classified by a self-report questionnaire. Alcohol consumption was classified by self-report as current, former, and never drinker. More information about subject recruitment and study measurements are available elsewhere [32].

Genotyping for linkage and follow-up association studies

Genomic DNA samples from MA family members were extracted from whole blood using standard methods, and used for genotyping of 366 microsatellite markers on 22 autosomes (9.4 cM average spacing) by the NHLBI Mammalian Genotyping Service of the Marshfield Medical Research Foundation. For association studies to follow-up the chromosome 15q linkage signal, we selected nine genes based on location relative to a major QTL on chromosome 15q, as well as involvement in pathways of BP regulation and nicotine metabolism (Gene Ontology and KEGG databases). For genotyping, we selected tag SNPs ($r^2 > 0.8$) from the International HapMap Project (Caucasians). In addition, we included functional SNPs (nonsynonymous and promoter SNPs) and additional SNPs that improved gene coverage (NCBI dbSNP). Multiplexed SNP genotyping used the SNPlex platform (Applied Biosystem) according to recommended protocols. We excluded SNPs with low minor allele frequencies (MAF<0.05), call rates < 90%, or lack of fit with Hardy-Weinberg expectations.

Statistical Analysis

Continuous values of SBP and DBP were adjusted for significant covariates including age, age^2, gender, BMI, diabetes, hypertension medication, and alternate blood pressure. Bivariate analysis (SBP and DBP) used the same adjustments except for alternate blood pressure. HT status was used as a discrete variable after adjustment for age, gender, BMI, and diabetes. We used four different strategies to control for the effect of antihypertensive treatment on the SBP and DBP measures. First, we statistically adjusted for HT medication by adding it to the model as a covariate. Second, we used HT status (subjects on HT medication classified as HT) as a discrete variable in addition to the continuous variables of SBP and DBP. Third, we added 10 mmHg to the value of SBP and 5 mmHg to the value of DBP in individuals who were taking HT medication. Fourth, we reran linkage analyses after excluding individuals on HT medication (only in the combined group). Many other covariates (alcohol consumption, LDL, HDL, total cholesterol, triglyceride, education) were tested for their effects on HT status and BP traits, but none of them significantly improved the models. Variance component methods implemented in the computer package SOLAR (version 2.1.4) were used for linkage analysis. Linkage was assessed by fitting a polygenic model that does not include genetic marker information for comparisons with models that include genotype data at a specific marker (two point analysis) or across the chromosome (multipoint analysis). Linkage analysis was performed separately for smokers, non-smokers, and the combined group. We considered evidence for linkage as significant (LOD≥3 for univariate analysis, LOD≥4 for bivariate analysis), suggestive (univariate LOD≥2, bivariate LOD≥2.87), or tentative (univariate LOD≥1.3, bivariate LOD≥2.06) [33]. We tested for GxS interactions at QTLs by comparing standard deviations for smokers versus non-smokers at loci with LOD scores that differed between the groups.

SNP association studies to follow-up the major QTL on chromosome 15q used Generalized Estimation Equation (GEE) regression methods with additive genetic models after adjustment for the same covariates as linkage analysis. GEE was performed separately for smokers, non-smokers, and the combined group with SAS 9.1 using proc genmod and exchangeable working correlation matrices. Corrections for multiple testing were based on false discovery rates (FDR) [34]. In addition, SNP association analyses used the Bayesian Quantitative Trait Nucleotide (BQTN) method as incorporated in SOLAR [35]. BQTN is an extension for the variance component method that evaluates the effect of every SNP individually, then every pair of SNPs, and then every combination of increasing numbers of SNPs. BQTN uses Bayesian model selection to average over the best models to avoid multiple testing problems and give the posterior probability of each SNP being the functional SNP. Conditional linkage analysis used the associated SNPs as covariates for reruns of the linkage analysis across the implicated region,

with reduction of LOD scores providing a measure of the SNP contributions to the linkage signal.

RESULTS

General characteristics of the MA population from GENOA are shown in Table 1. Distributions of subjects with type II diabetes and hypertension were similar between smokers and non-smokers. We found no significant differences between smokers and non-smokers for HT treatment. Smokers had significantly higher DBP and lower BMI compared to non-smokers, but no significant differences were found for SBP.

Genome wide linkage scan for GxS interactions on HT and BP measures

Figure 1 shows the multipoint LOD scores for SBP, DBP, bivariate analysis (SBP and DBP), and HT status in smokers, non-smokers, and the combined group. Overall, SBP provided the best evidence for linkage in MA families relative to the other traits. Figure 2 shows multipoint LOD curves for five major QTLs for SBP, and Table 2 shows their locations and maximum LOD scores for smokers, non-smokers and the combined group. The strongest evidence for linkage was found for SBP on chromosome 17 (LOD=4.2) in the combined group (Figure 2, Table 2), and there was no evidence for GxS interactions ($p = 0.48$). However, other SBP QTLs were detected mainly in smokers or non-smokers. QTLs on chromosome 15 (LOD= 3.37) and chromosome 6 (LOD= 2.06) were found mainly in non-smokers, while QTLs on chromosome 20 (LOD=2.45) and chromosome 7 (LOD= 1.47) were found mainly in smokers (Figure 2, Table 2). Evidence for GxS interactions was very strong for the QTLs on chromosome 15 ($p = 0.0004$) and chromosome 7 ($p = 0.009$). The QTLs on chromosomes 15, 17, and 20 remained significant after correction for multiple testing (FDR at 5% level). In general, bivariate analyses yielded smaller linkage signals and did not detect any QTLs that were not detected by univariate analysis, despite strong genetic correlation between SBP and DBP (0.78 for smokers, 0.85 for non-smokers, and 0.79 for the combined group). Our analyses used adjustment for antihypertensive medication as a covariate for SBP and DBP. We also tried other approaches such as addition of 10 mmHg to the value of SBP and 5 mmHg to DBP in individuals who were taking HT medication, but the results remained unchanged by this correction. We also reran linkage analyses after excluding individuals on HT medication in the combined group, which slightly lowered LOD scores for QTLs on chromosomes 17 (LOD=1.6) and 20 (LOD=1.5) due to the loss of power consequent to using fewer individuals.

Follow-up SNP association studies for the major QTL for SBP on chromosome 15q

Our first priority for follow-up was the major SBP QTL on chromosome 15q that showed significant evidence for linkage only in non-smokers (LOD=3.37), and that showed strong evidence for GxS interaction ($p = 0.0004$) (Figure 2, Table 2). The linkage signal on chromosome 15 spans approximately 40 cM (15q22.2 to 15q26.3). Of a total of 436 genes in this region, we chose 9 high priority genes based on close proximity to the maximal LOD score and involvement in pathways relevant to BP regulation and nicotine metabolism. Table 3 lists these genes, as well as their chromosomal location and functional pathways. For each gene, we selected tag SNPs from HapMap (total 27 tag SNPs) providing coverage of 246 SNPs ($r^2>0.8$), as well as an additional 28 SNPs with functional attributes or that enhanced gene coverage (NCBI dbSNP). Table 4 presents information on these 55 SNPs including chromosomal position, gene region, alternate alleles, and MAF.

Figure 3 presents the results of association analysis using GEE and BQTN in the three BP related traits in all groups. For HT in non-smokers, GEE identified 13 significant SNPs, four of which (ADAMTS17_rs8027190 C/T, CHRNA3_rs1317286 A/G, CHRNA5_rs16969968 A/G, and CHRNA5_rs951266 C/T) were confirmed using BQTN (posterior functional

probabilities of 0.73, 0.77, 0.42, and 0.40 respectively). Three of these confirmed SNPs showed associations with HT in the combined group using GEE but not BQTN, and none showed associations in smokers. For HT in smokers, there was only one SNP (SLCO3A1_rs207961 C/G) that showed significant associations for both GEE and BQTN with posterior functional probability of 0.61, which was not significant in non-smokers or in the combined group.

For DBP, GEE identified three SNPs in two genes (ADAMTS17_rs2573652 C/T, IGF1R_rs4966035 A/G and IGF1R_rs1879613 C/T) that showed significant associations in non-smokers, but not in smokers. However, none of these SNPs were significant using BQTN.

For SBP, GEE identified four SNPs in three genes (ANPEP, IGF1R, SLCO3A1) that showed significant associations in non-smokers, the group which yielded the original SBP QTL on chromosome 15q. BQTN confirmed associations for three of these SNPs (ANPEP_rs753362 C/G, IGF1R_rs7166287 C/T, and SLCO3A1_rs1983350 A/T) with posterior functional probabilities of 0.67, 0.63, and 0.55 respectively. These three SNPs did not show associations with SBP in smokers, and only IGF1R_rs7166287 C/T was associated with SBP in the combined group. IGF1R_rs7166287 C/T was also significantly associated with HT status in non-smokers in GEE analysis.

Allelic effects of chromosome 15q candidate genes associated with SBP in non-smokers

We investigated the allelic effects of the three SNPs in candidate genes (ANPEP, IGF1R, SLCO3A1) on chromosome 15q that showed associations with SBP in non-smokers. Individually, the minor alleles for ANPEP_rs753362 C/G and IGF1R rs7166287 C/T were each associated with 1.5 mmHg increase in mean SBP value, while the A allele for SLCO3A1 rs1983350 A/T was associated with an increase of 1.9 mmHg. We also investigated their cumulative effects on SBP by grouping individuals according to the number of risk alleles that they carry, with a maximum of six risk alleles (homozygotes for risk alleles at all three loci). Figure 4 shows the cumulative effects on mean SBP values with increase in the number of the risk alleles carried by GENOA subjects. The combined effect of the risk alleles for the three SNPs was to raise mean values of SBP by approximately 7 mmHg (p=0.01).

DISCUSSION

We used genome-wide linkage analyses to identify QTLs that mediate effects of gene by smoking (GxS) interaction on hypertension and blood pressure measures in MA families from GENOA. We identified QTLs only in smoker or non-smoker groups that showed strong GxS interactions. A major QTL for SBP on chromosome 15q was found only in non-smokers (LOD=3.37) and showed strong GxS interactions (p=0.0004) (Figure 2, Table 2). In addition, we found a QTL for SBP on chromosome 7q only in smokers (LOD=1.47) that showed strong GxS interactions (p=0.009). It is not clear why SBP emerged as the principal trait that yielded major QTLs in linkage analyses, but previous studies have reported similar results. Genome wide linkage scans for both SBP and DBP between 1999 and 2004 reported 25 QTLs linked to SBP versus only 10 QTLs linked to DBP [1,2]. Perhaps DBP is controlled by genes with smaller effects compared to SBP, presenting a greater challenge for detection of contributing genes using linkage analysis. It is difficult to explain why these QTLs appear only in smokers or non-smokers. Perhaps the QTLs effect on BP in non-smoking individuals is somehow masked in smokers, in which a different array of genes may regulate BP due to the complex chemical and physiological impacts of smoking.

Many of the chromosomal regions that showed linkage with SBP in MA families have also shown evidence for linkage with BP related traits in previous studies. The chromosome 17q region containing a major QTL for SBP (LOD = 4.26 at 89 cM) in the combined group had previously shown linkage for pulse pressure (LOD=3.6 at 89 cM) and BP (LOD=3.2 at 82 cM)

in MA families from GENOA [29,30]. Genome scans in other racial groups have identified linkage of chromosome 17q with BP related traits including SBP in European Americans from the Framingham Heart Study (LOD=4.7 at 67 cM and LOD=2.2 at 94 cM) and HT in Chinese Han families (LOD=1.8 at 89 cM) [36,37].

Since the major goal for this study was to identify QTLs for GxS interaction, we were particularly interested in the major QTL for SBP on chromosome 15q (LOD = 3.37 at 110 cM) found only in non-smokers. This region of chromosome 15q has shown linkage in previous studies of EA families (Rochester MN) using trivariate analysis (SBP, DBP, BMI) (LOD = 2.99 at 87 cM) [33], and for SBP in GENOA EA families (P=0.003 at 97 cM) [38]. Linkage in EA families has also been found in the Framingham Heart Study for SBP (−log p-value = 6.68 at 129 cM) [39] and pulse pressure (LOD = 2.4 at 103 cM) [40], as well as for HT in the Family Heart Study (LOD = 2.94 at 122 cM) [41]. Interestingly, this region of chromosome 15q with strong evidence for G x S interaction in MA also showed linkage with smoking rate in EA families from the Framingham Heart study (p=0.003 at 127 cM) [42].

To begin identification of genes and polymorphisms that underlie the major QTL for effects of GxS interaction on SBP, we selected nine candidate genes involved in processes related to BP and smoking for association analyses (tag SNPs and functional SNPs) in the linked region of chromosome 15q (Table 3). Overall, the largest number of associations occurred in non-smokers, the same group in which the chromosome 15q QTL for SBP was found. We were particularly interested in genes with SNPs that showed significant associations with SBP in non-smokers using both regression and Bayesian analytic approaches (GEE and BQTN), and that did not show associations in smokers. Three genes contained SNPs that met these criteria including ANPEP (alanyl aminopeptidase), IGF1R (insulin-like growth factor 1 receptor), and SLCO3A1 (solute carrier organic anion transporter family, member 3A1). SNP rs753362 C/G is located in intron 15 of ANPEP that encodes an aminopeptidase N ectoenzyme which is expressed in the kidney and the brain. ANPEP plays a role in the renin-angiotensin system (RAS) by converting angiotensin III to angiotensin IV, which in turn is involved in BP/HT regulation [43].We also found associations of rs7166287 C/T in intron 3 of IGF1R with SBP in non-smokers. IGF1R is involved in the insulin signaling pathway, which makes it a strong candidate for BP regulation given the strong relationship between HT and diabetes [44]. In addition, we found associations of rs1983550 A/T in intron 3 of SLC03A1 with BP in non-smokers. SLCO3A1 is a member of the family of organic anion transporting polypeptides which are responsible for transferring organic solutes including some drugs and xenobiotics, suggesting potential roles for metabolism of cigarette smoke and BP regulation [45]. Each of these SNPs have individual allelic effects on SBP, and cumulatively are associated with a significant increase in SBP of approximately 7 mmHg (p=0.01) (Figure 3). These three genes were not chosen from a particular metabolic pathway, limiting speculation about their joint effects on blood pressure. In general, much remains unknown concerning the physiological connections of smoking with blood pressure regulation. However these genes were chosen due to their location under linkage peaks and function related to BP and nicotine metabolism, and may represent novel targets for further studies of GxS interaction using statistical and experimental approaches.

Our finding of SBP associations in non-smokers with SNPs in three genes on chromosome 15q (ANPEP, IGF1R, and SLC03A1) provides evidence for their contributions to the major QTL for SBP on chromosome 15q in non-smokers that showed strong GxS interactions. We examined this relationship with the SBP linkage signal by conditional linkage analysis that used combined genotypes for the three associated SNPs as covariates for linkage analysis. In comparison with original linkage results, phenotypic adjustment for the combined SNP genotypes at the three loci reduced the LOD score for SBP linkage on chromosome 15q from 3.37 to 3.08 (8.7% reduction). We would have expected a more dramatic reduction of the LOD

score if the three loci were solely responsible for the major QTL for SBP in non-smokers. Additional fine mapping studies will be required to identify the gene(s) in the region that are responsible for the QTL for SBP on chromosome 15q in non-smokers.

A potential limitation of this study is enrichment of subjects with type II diabetes resulting from the GENOA recruitment strategy that selected pedigrees with at least two diabetic sibs. We have attempted to address this ascertainment bias by adjustment for diabetes status in statistical analyses. Overall, our findings may be particularly relevant to diabetic individuals of Mexican-American descent.

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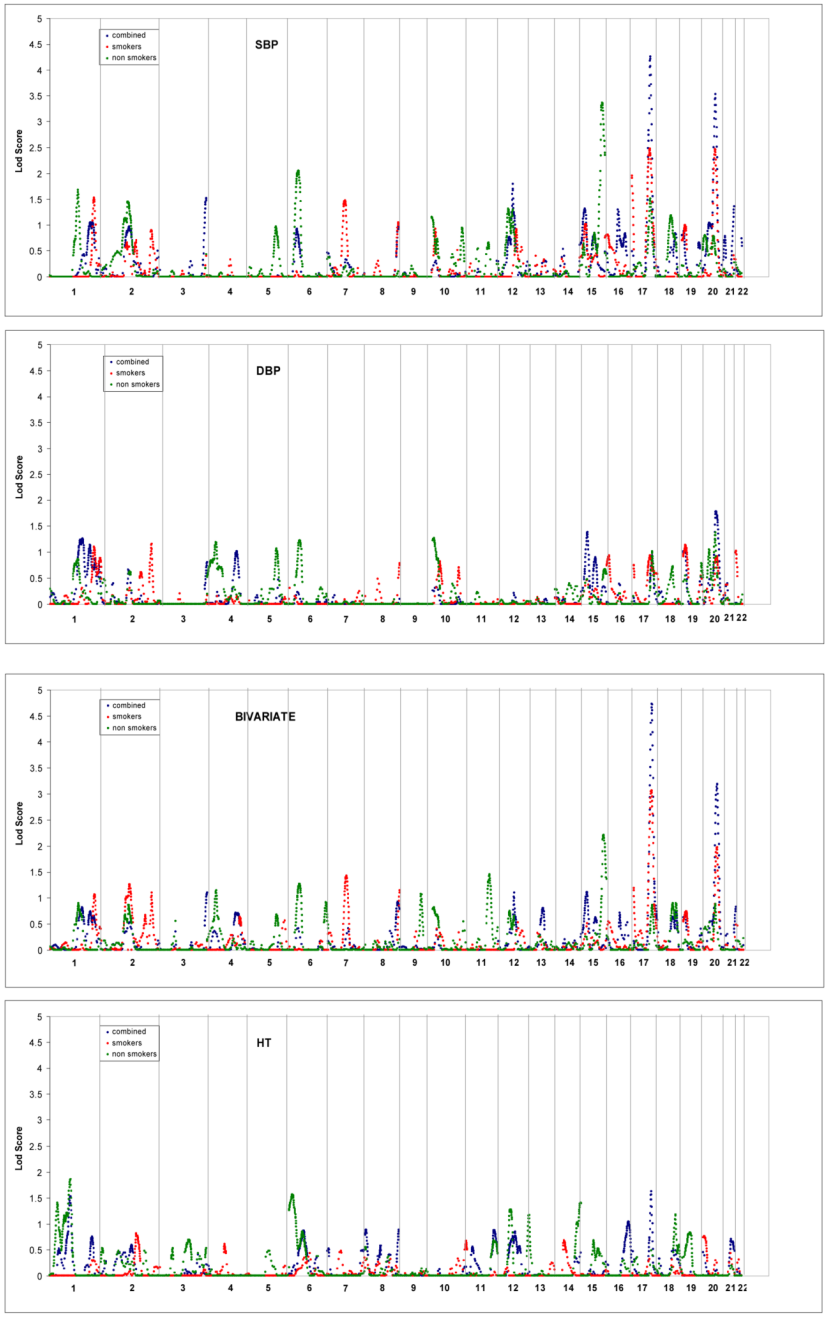

Figure 1.
Multipoint LOD curves for hypertension (HT), systolic blood pressure (SBP), diastolic blood pressure (DBP), bivariate analysis of SBP and DBP in smokers (red dots), non-smokers (green dots), and the combined group (blue dots) across all 22 autosomes.

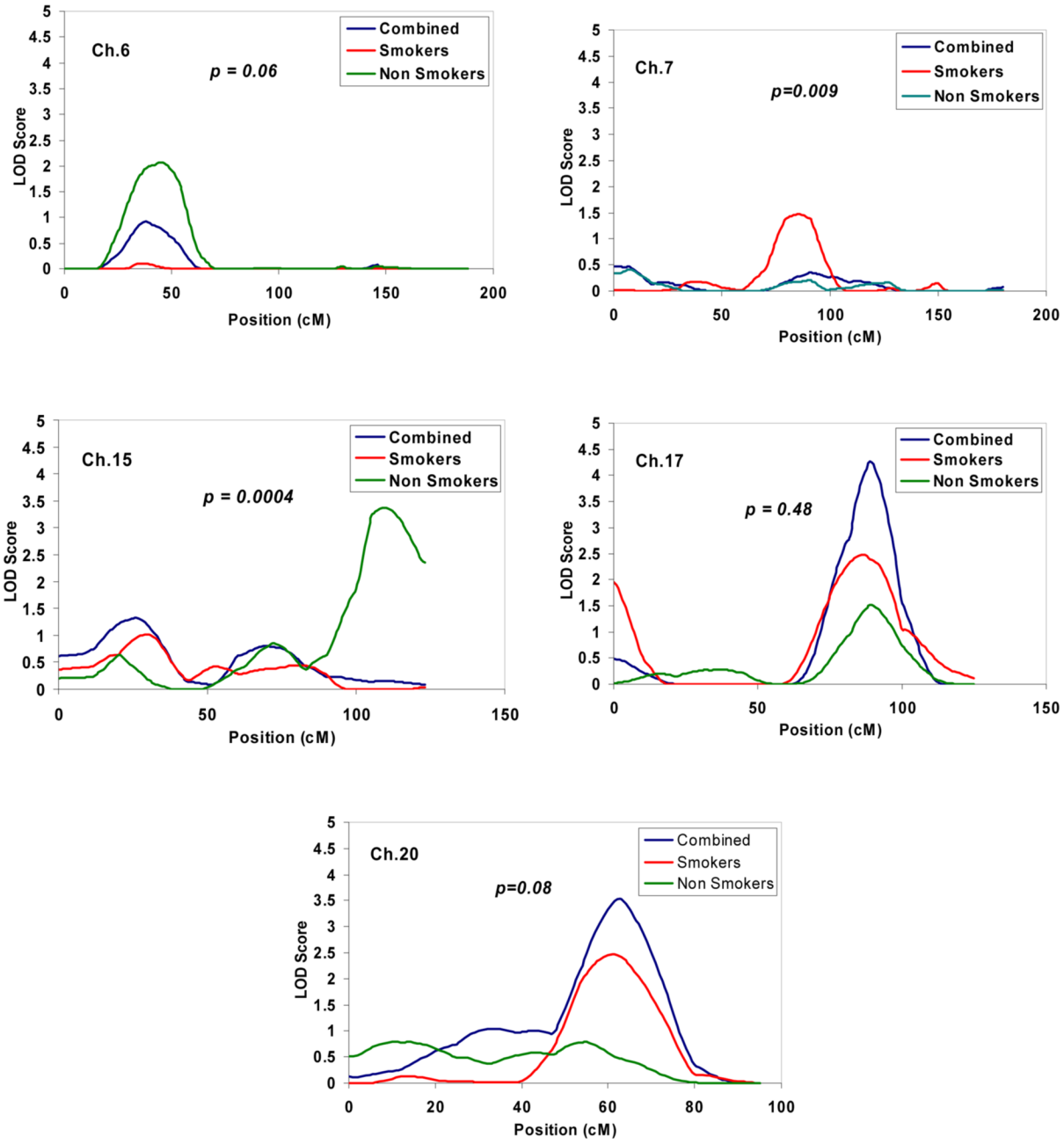

Figure 2.
Multipoint LOD curves on chromosomes 6, 7, 15, 17, and 20 for SBP in smokers (red dots), non-smokers (green dots), and the combined group (blue dots).

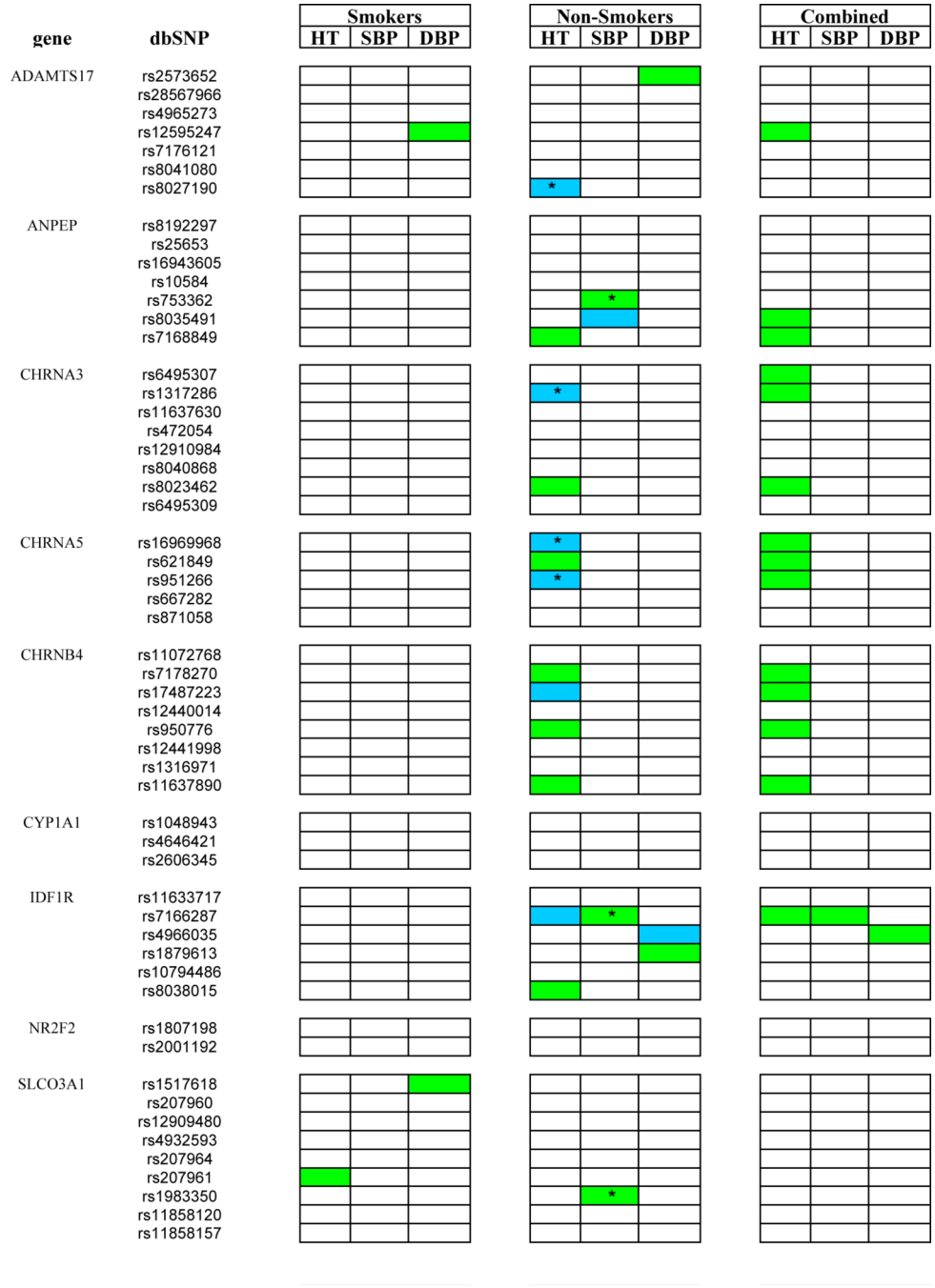

Figure 3.

Results of association analysis for SNPs in nine candidate genes on chromosome 15q for smokers, non-smokers, and the combined group. Green cells show significant associations using GEE with $p<0.05$, blue cells show significant associations using GEE with $p<0.01$, and cells marked with (*) show significant associations using BQTN.

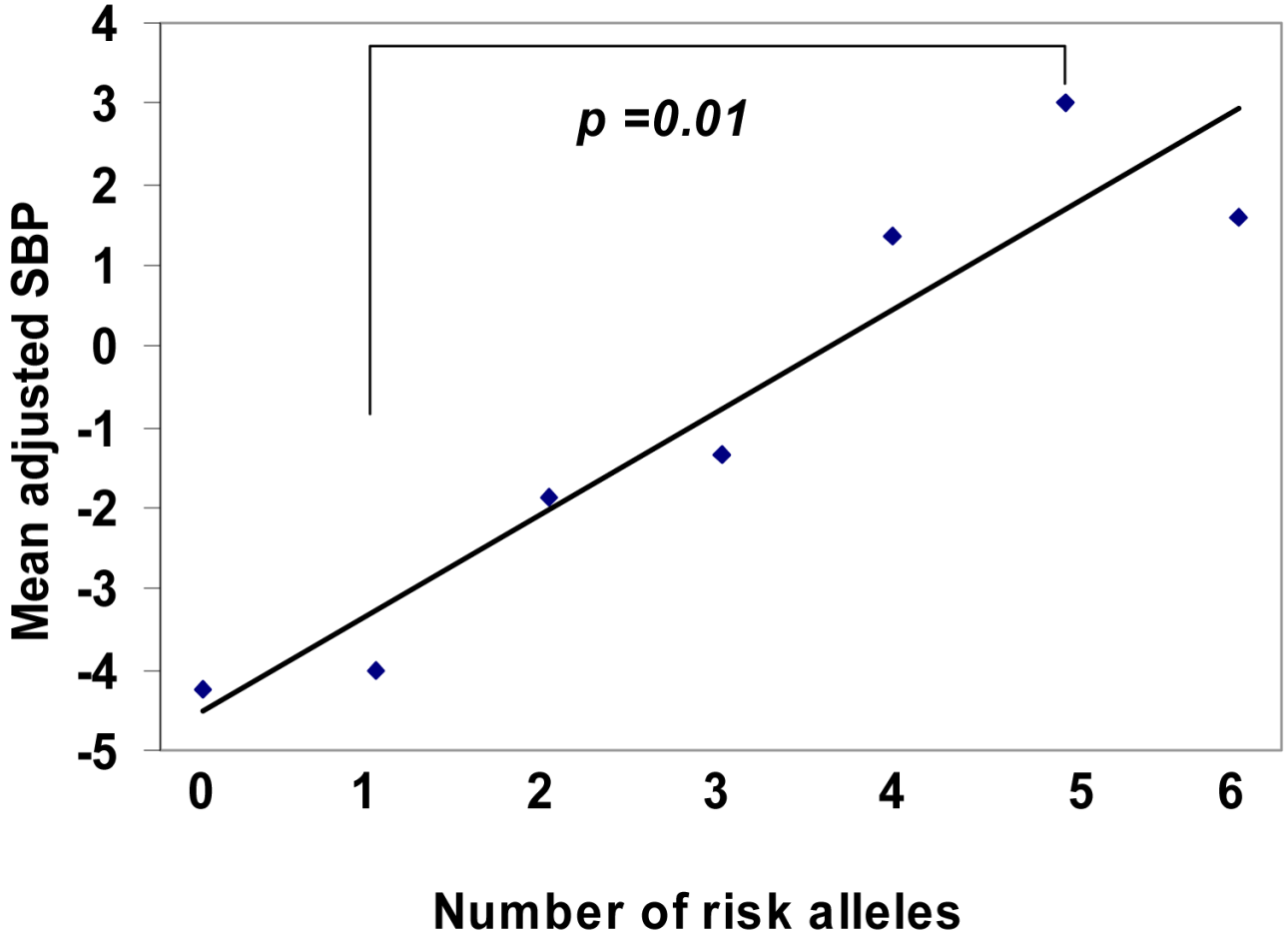

Figure 4.
The cumulative effect of risk alleles for ANPEP_rs753362 C/G, IGF1R_rs7166287 C/T, and SLCO3A1_rs1983350 A/T on mean adjusted values of SBP in non-smokers. The numbers of individuals with 0, 1, 2, 3, 4, 5, and 6 risk alleles are 4, 38, 164, 299, 278, 135, and 29, respectively. The best fitting trend line and p-value from t-tests between the genotypic group carrying 1 risk allele and those with 5 risk alleles are shown.

Table 1

General characteristics for smokers, non-smokers, and the combined group (means ± SD for continuous variables and prevalence for categorical variables).

	Smokers	Non-smokers	Combined
No. of subjects*	672	977	1649
No. of families*	345	405	420
Male (%)*	69.2	21.6	40.9
Hypertension (%)	47.7	50	49.6
Diabetes (%)	59.9	61.7	60.9
HT treatment %	21.7	24	23.3
SBP	131±22	130±22	130±22
DBP*	72±10	70±10	71±10
BMI*	29±5	31±6	30±6
Age	56±11	54±12	55±12

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index

*Significant difference between smokers and non-smokers ($p \leq 0.0001$).

Table 2

Maximum LOD scores for QTLs with the strongest evidence for linkage in smokers, non-smokers, and the combined group.

Ch	cM	Nearest marker	From marker	To Marker	trait	Smoker	Non smoker	combined
6	45	D6S2439	D6S2434	D6S2410	SBP	0.03	2.06**	0.80
					DBP	0.00	1.22	0.18
					BI	0.03	1.27	0.35
					HT	0.12	0.53	0.38
7	85	D7S2204	D7S1818	D7S821	SBP	1.47*	0.18	0.27
					DBP	0.04	0.05	0.00
					BI	1.37	0.01	0.21
					HT	0.04	0.00	0.00
15	110	D15S657	D15S655	D15S642	SBP	0.00	3.37***	0.15
					DBP	0.00	0.62	0.34
					BI	0.01	2.20	0.09
					HT	0.00	0.24	0.06
17	89	D17S2193	D17S2180	D17S784	SBP	2.39**	1.51*	4.26***
					DBP	0.64	1.00	0.93
					BI	2.96**	0.86	4.71***
					HT	0.06	0.47	1.62*
20	62	D20S481	D20S470	D20S171	SBP	2.45**	0.49	3.53***
					DBP	0.92	0.59	1.71*
					BI	1.98	0.39	3.19**
					HT	0.34	0.03	0.17

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BI: Bivariate; HT: Hypertension

* Tentative evidence for linkage (univariate LOD≥1.3, bivariate LOD≥2.06)

** Suggestive evidence for linkage (univariate LOD≥2, bivariate LOD≥2.87)

*** Significant evidence for linkage (univariate LOD≥3, bivariate LOD≥4)

Table 3

Identity, location, and function of nine candidate genes selected for follow-up fine mapping of the chromosome 15q QTL for SBP with strong GxS interactions

NCBI Gene ID	Ch. Location	Gene symbol	Gene name	Function/Pathway
1543	72798943	CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	ion and heme binding, fatty acid, drug and xenobiotic metabolism.(other member in this family play a role in the aldosterone production and cause rare form of HT)
1138	76644961	CHRNA3	Nicotinic cholinergic receptor, alpha polypeptide 3	ion transport, signal transduction
1136	76674706	CHRNA5	Nicotinic cholinergic receptor, alpha polypeptide 5	ion transport, signal transduction
1143	76703691	CHRNB4	Nicotinic cholinergic receptor beta polypeptide 4	ion transport, signal transduction
290	88129130	ANPEP	Alanyl aminopeptidase	angiogenesis, aminopeptidase activity (expressed in the kidney), regulate vasopressin release
28232	90197950	SLCO3A1	solute carrier organic anion transporter family, member 3A1	ion transport
7026	94674950	NR2F2	nuclear receptor subfamily 2, group F, member 2	signal transduction, lipid metabolism
3480	97010288	IGF1R	Insulin-like growth factor 1 receptor	insulin signaling, calcium signaling
170691	98331993	ADAMTS17	ADAM (a disintegrin and metaloproteinase) metallopeptidase with thrombospondin type 1 motif, 17	ion binding

Table 4

Identity, location, number of SNPs tagged (Tag #), and minor allele frequencies (MAF) in the nine candidate genes for fine mapping of the chromosome 15q QTL for SBP in non-smokers.

Gene	dbSNP	Alleles	Ch. position	Region/Function	Tag #	MAF
ADAMTS17	rs2573652	C/T	98332137	Exon 23/S1094N	3	0.409
ADAMTS17	rs28567966	A/G	98510368	Exon_11/T482M		0.092
ADAMTS17	rs4965273	A/G	98402341	Intron 19	30	0.419
ADAMTS17	rs12595247	C/T	98359212	Intron 19	11	0.489
ADAMTS17	rs7176121	C/T	98662843	Intron 4	9	0.353
ADAMTS17	rs8041080	C/T	98661407	Intron 4	9	0.473
ADAMTS17	rs8027190	C/T	98457727	Intron 15	7	0.296
ANPEP	rs8192297	A/G	88145356	Exon_12/ M603I		0.391
ANPEP	rs25653	A/G	88150562	Exon_2/ Q86R		0.375
ANPEP	rs16943605	C/T	88137377	Intron_15	12	0.255
ANPEP	rs10584	C/G	88129555	Exon 22/ UTR	2	0.438
ANPEP	rs753362	C/G	88141538	Intron 15		0.445
ANPEP	rs8035491	A/G	88143357	Intron 14		0.331
ANPEP	rs7168849	A/G	88147231	Intron 10		0.350
CHRNA3	rs6495307	C/T	76677376	Intron 6	8	0.233
CHRNA3	rs1317286	A/G	76683184	Intron 5	3	0.229
CHRNA3	rs11637630	A/G	76686774	Intron 5	9	0.463
CHRNA3	rs472054	C/T	76675049	Exon 8/UTR		0.196
CHRNA3	rs12910984	A/G	76678682	Intron 6		0.467
CHRNA3	rs8040868	C/T	76698236	Exon_3		0.290
CHRNA3	rs8023462	C/T	76701789	Promoter		0.169
CHRNA3	rs6495309	C/T	76702300	Promoter		0.492
CHRNA5	rs16969968	A/G	76669980	Exon_6/ N398D		0.222
CHRNA5	rs621849	A/G	76659916	Intron 2	14	0.228
CHRNA5	rs951266	C/T	76665596	Intron 3	4	0.227
CHRNA5	rs667282	C/T	76650527	Intron 2	3	0.463
CHRNA5	rs871058	A/G	76645546	Intron 2		0.187
CHRNB4	rs11072768	G/T	76716533	Intron 2		0.497
CHRNB4	rs7178270	C/G	76708132	Intron 6		0.207

Gene	dbSNP	Alleles	Ch. position	Region/Function	Tag #	MAF
CHRNB4	rs17487223	C/T	76711042	Intron 3		0.269
CHRNB4	rs12440014	C/G	76713781	Intron 3		0.499
CHRNB4	rs950776	C/T	76713073	Intron 3		0.151
CHRNB4	rs12441998	A/G	76716427	Intron 2		0.492
CHRNB4	rs1316971	A/G	76717565	Intron 2		0.493
CHRNB4	rs11637890	C/G	76722474	Promoter		0.170
CYP1A1	rs1048943	A/G	72800038	Exon 8/ V462I		0.237
CYP1A1	rs4646421	C/T	72803245	Intron 1/ UTR		0.299
CYP1A1	rs2606345	A/C	72804229	Intron 1/ UTR		0.464
IGF1R	rs11633717	C/T	97090555	Intron 3	14	0.255
IGF1R	rs7166287	C/T	97090598	Intron 3	13	0.446
IGF1R	rs4966035	A/G	97246985	Intron 3	7	0.400
IGF1R	rs1879613	C/T	97251880	Intron 3	5	0.295
IGF1R	rs10794486	A/G	97119058	Intron 3	4	0.297
IGF1R	rs8038015	C/T	97080797	Intron 3	8	0.400
NR2F2	rs1807198	C/T	94679298	Intron 4	2	0.346
NR2F2	rs2001192	A/G	94679441	Intron 4		0.288
SLCO3A1	rs1517618	C/G	90448649	Exon_5/ D294E		0.113
SLCO3A1	rs207960	G/T	90455723	Intron 5	10	0.136
SLCO3A1	rs12909480	C/T	90317473	Intron 3	13	0.230
SLCO3A1	rs4932593	A/G	90320402	Intron 3	12	0.335
SLCO3A1	rs207964	A/G	90453177	Intron 5	12	0.114
SLCO3A1	rs207961	C/G	90454608	Intron 5	10	0.371
SLCO3A1	rs1983350	A/T	90433579	Intron 3	12	0.196
SLCO3A1	rs11858120	A/T	90196267	Promoter		0.251
SLCO3A1	rs11858157	A/G	90196402	Promoter		0.234