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Autosomal Dominant Orthostatic Hypotensive Disorder Maps to Chromosome 18q

Anita L. DeStefano,[1,2] Clinton T. Baldwin,[3] Michael Burzstyn,[2,*] Irene Gavras,[2] Diane E. Handy,[2] Oscar Joost,[3] Timothy Martel,[3] Michael Nicolaou,[1] Faina Schwartz,[2] David H. P. Streeten,[4] Lindsay A. Farrer,[1,2] and Haralambos Gavras[2]

Departments of [1]Neurology, Epidemiology and Biostatistics, and [2]Medicine and [3]Center for Human Genetics, Boston University School of Medicine, Boston; and [4]Department of Medicine, State University of New York Health Science Center, Syracuse

Summary

Familial orthostatic hypotensive disorder is characterized by light-headedness on standing, which may worsen to syncope, palpitations, and blue-purple ankle discoloration, and is accompanied by a marked decrease in systolic blood pressure, an increase in diastolic pressure, and tachycardia, all of which resolve when supine. We ascertained three families in which this disorder is inherited as an autosomal dominant trait with reduced penetrance. A genomewide scan was conducted in the two largest families, and three regions with multipoint LOD scores >1.5 were identified. Follow-up of these regions with additional markers in all three families yielded significant evidence of linkage at chromosome 18q. A maximum multipoint LOD score of 3.21 in the three families was observed at D18S1367, although the smallest family had negative LOD scores in the entire region. There was significant evidence of linkage in the presence of heterogeneity at 18q, with a maximum LOD score of 3.92 at D18S1367 in the two linked families. Identification of the gene responsible for orthostatic hypotensive disorder in these families may advance understanding of the general regulatory pathways involved in the continuum, from hypotension to hypertension, of blood pressure.

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Address for correspondence and reprints: Dr. Clinton T. Baldwin, Center for Human Genetics, Boston University School of Medicine, 80 East Concord Street, W408, Boston, MA 02118. E-mail: cbaldwin@bu.edu

[*] Present affiliation: Department of Medicine, Hadassah University Hospital, Jerusalem.

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Introduction

The regulation of blood pressure (BP) is a multifactorial process that involves numerous biological pathways that maintain a constant equilibrium between vasoconstricting and vasodilating factors. Arterial hypertension is the result of chronic tilting of this balance toward vasoconstriction, owing to an interplay of genetic and environmental factors, and, consequently, results in an increase in peripheral resistance. Hypotension is usually an acute clinical syndrome that follows loss of blood volume, endocrine or vasomotor disorders, or cardiac (rhythm or function) disturbances. Hypotension can also be a chronic condition that stems from autonomic dysfunction and is either idiopathic or secondary to a variety of systemic diseases, such as diabetes mellitus or amyloidosis (Robertson et al. 1992). In rare cases, hypotension is part of the constellation of abnormalities that define a variety of familial disorders that are a result of specific genetic defects, such as the monoamine oxidase deficiency owing to an X-chromosome deletion (Sims et al. 1989). The majority of studies to identify genes that influence BP have focused on hypertensive individuals, but the isolation of genes in familial disorders characterized by hypotension is equally important to the dissection of the process of BP regulation.

In 1972, Streeten and colleagues reported a novel familial orthostatic hypotensive syndrome (MIM 143850; Streeten et al. 1972). Five patients from four families were described as having orthostatic intolerance that ranged in severity from light-headedness to syncope, owing to a profound fall in systolic BP (SBP) and a rise in diastolic BP, that in many cases was associated with flushing, palpitations, leg edema, and/or varying purplish discoloration. The original clinical evaluation ruled out known causes of orthostatic hypotension and attributed the symptoms to hyperbradykininism. All patients had affected first-degree relatives, indicating a genetic basis for the disorder.

Extended pedigree information and DNA samples were obtained for three of the families in the original

report, and linkage analyses were conducted. We report here the identification of a chromosomal region on 18q, which demonstrates significant evidence of linkage to the disorder in these families.

Families and Methods

Families

Members of the four families from which the five patients described by Streeten et al. (1972) originated were contacted directly and were asked about the presence of symptoms, including dizziness and/or purple discoloration of legs on standing, their use of medication for BP regulation, and their ability to engage in physical activity. Information on minor children was provided by a parent. Medical records from prior exams (by D.H.P.S.) were available for the majority of patients who self-reported an affected status. In addition, local physicians were questioned regarding their patients' symptoms, BP response to orthostasis, and treatment. One family was excluded from this study because its current physician observed no important changes in BP or in heart rate during orthostasis, despite the presence of other symptoms. For the remaining three families, DNA samples were obtained from 38 individuals, including 14 affected individuals and 1 obligate carrier (fig. 1). Clinical measurements for patients are shown in table 1. For linkage analysis, individuals were classified as affected if an excessive orthostatic drop in SBP (>18 mm/Hg) and rise in heart rate were observed. Individuals for whom detailed clinical information was not available were classified as affected if they reported orthostatic symptoms. Individual 5066 is not currently being treated and was initially reported as unaffected. Reevaluation of his clinical status revealed that he experiences light-headedness on standing; however, he declines treatment. In this analysis he is classified as affected. Individual 5052 experiences a significant drop in SBP on standing and is, therefore, classified as affected, but she experiences no symptoms. She is a high-level athlete, and it is possible that her physical condition enables her to compensate for her BP changes. A single nonpenetrant individual (5176) with an affected parent and offspring was observed.

Genotyping

Peripheral blood was obtained from the subjects in accordance with institutional guidelines for human subjects, and DNA was isolated from blood leukocytes by the proteinase K-SDS method (Sambrook et al. 1989). A series of 200 highly polymorphic tri- and tetranucleotide microsatellite markers, spaced an average of 20 cM apart, were typed in the two largest families (Sheffield et al. 1995). To make efficient use of laboratory resources, the third family was typed only for markers in the regions of interest that had been identified in the two larger families. A standard amplification reaction (10 μl; Saiki et al. 1988) was performed, with 40 ng genomic DNA and 1.0 pmol of each primer. Cycling parameters consisted of 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 60°C for 30 s, and 72°C for 30 s. Prior to amplification, one primer was end-labeled with ^{32}P and polynucleotide kinase (New England Biolabs), in order for the amplification product to be visible by autoradiography after application to a standard sequencing gel.

Linkage Analysis

For initial screening, multipoint parametric linkage was assessed using GENEHUNTER (Kruglyak et al. 1996) in the two families (BU134 and BU233) in which the genome scan was performed. An autosomal dominant mode of transmission with a reduced penetrance of 80% was assumed. Penetrance was not modeled as age dependent, because symptoms typically appear in childhood, and all unaffected children included in the analysis are older than the youngest onset age. Marker-allele frequencies were assumed to be equal, but this assumption had little influence on the linkage results, because nearly complete genotype information was available for the founders. Given the number of individuals in the largest family and computational limitations, two unaffected individuals (5067 and 5069) could not be included in the GENEHUNTER analysis. Since the sharing of alleles among affected individuals is most critical for identifying linkages in a reduced-penetrance model, no linked regions would be missed by exclusion of these two unaffected persons. The genetic information provided by them could have small effects on the LOD scores, however. To maximize the amount of genetic information extracted from the largest family, regions that yielded an aggregate LOD score $\geqslant 1.5$ were reanalyzed by VITESSE (O'Connell and Weeks 1995). This enabled the inclusion of all individuals when a limited number of markers in a specific chromosomal region was selected. Positive regions were further examined by the typing of additional markers in all three families. After completion of the genome screen, a DNA sample was obtained on an additional affected individual (7051). Therefore, LOD scores computed on the basis of the follow-up of positive regions reflect an additional meiotic event, as well as increased marker information. Model assumptions for VITESSE analyses were identical to those used in GENEHUNTER. The possibility of linkage heterogeneity was considered by performance of the admixture test, implemented in the program HOMOG (Ott 1991).

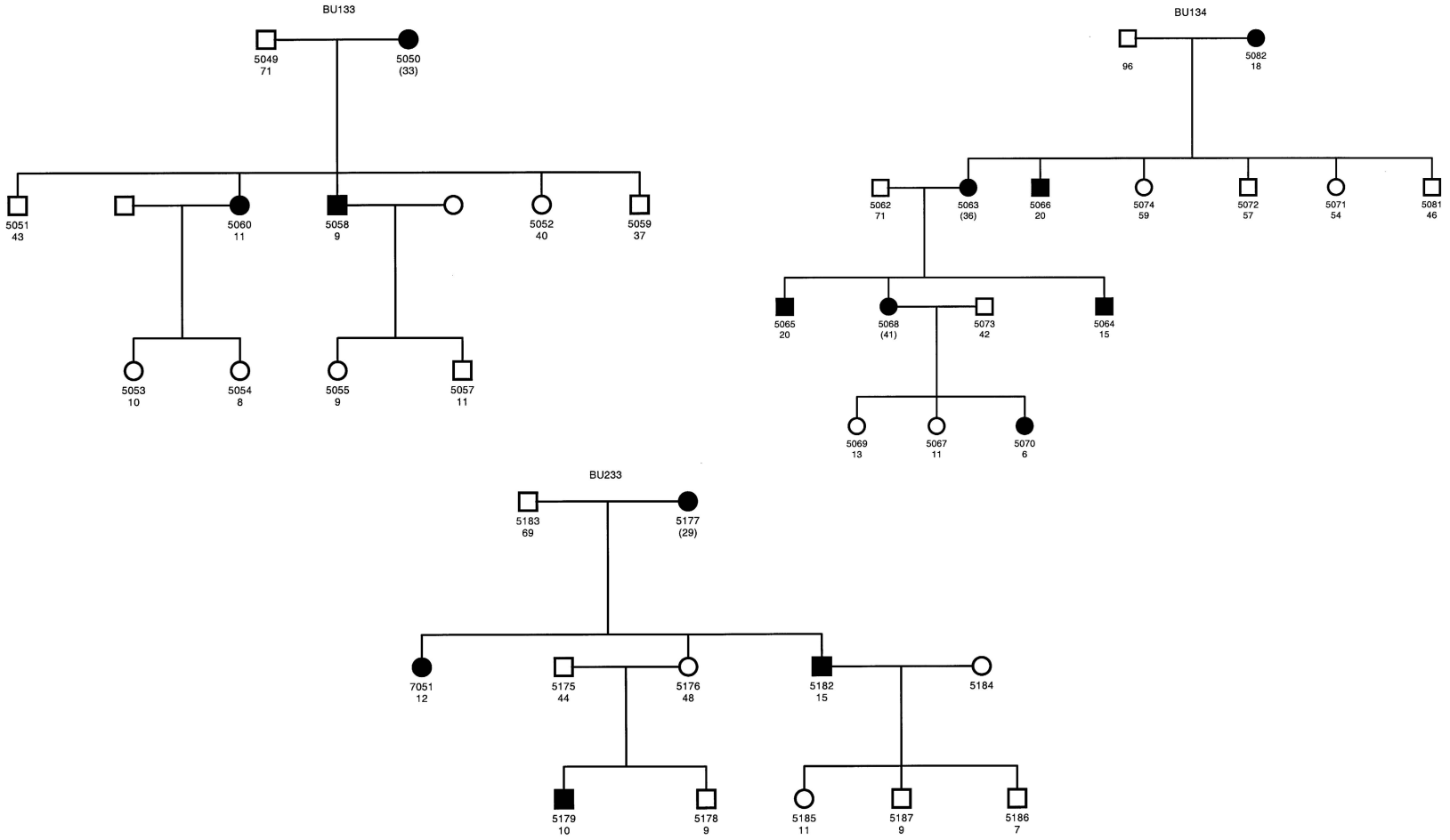

Figure 1 Pedigrees of three families with BP-regulation disorder. Identification numbers, which indicate that a DNA sample was obtained, are given under the symbols. Onset age (affected individuals) or current age (unaffected individuals) are shown. Values in parentheses are age at diagnosis for affected individuals for whom precise onset age could not be established.

Table 1

Clinical Information Obtained from Affected and Unaffected Family Members

Family and Individual	Blood Pressure(mm/Hg)		Heart Rate (bpm)[a]		Clinical Diagnosis[b]
	Lying	Standing	Lying	Standing	
BU133:					
5050	124/90	98/88	88	144	A
5051	124/50	122/84	64	84	N
5052	114/68	94/70	80	104	A
5058	108/58	90/82	72	104	A
5060	126/68	64/60	80	156	A
BU134:					
5063	118/70	100/74	54	72	A
5064	114/74	86/74	72	112	A
5065	136/70	102/90	68	108	A
5068	116/58	80/70	88	132	A
BU233:					
5177	154/86	128/100	76	116	A
5178	100/60	104/64	60	68	N
5182	118/76	80/74	76	164	A
5185	114/64	104/76	60	72	N
5186	90/52	90/60	72	88	N
5187	106/56	106/64	72	96	N
7051	115/72	84/76	70	108	A

[a]bpm = beats per minute
[b]A = affected; N = unaffected.

Results

Multipoint parametric LOD-score analysis excluded 1,512 cM by the conservative criterion of a LOD score <-2. An additional 705 cM had negative evidence for linkage (LOD score <-1). Three regions (13p, 3q, and 18q) showed suggestive evidence of linkage, on the basis of the criterion of an aggregate LOD score >1.5. Reanalysis of the positive regions, conducted by use of the VITESSE program, yielded a maximum multipoint LOD score (Z_{max}) of 2.19 at 13p, 2.21 at 3q, and 3.5 at 18q. These locations were investigated further by typing additional markers in all three families.

Saturation of the 18q region with additional markers resulted in an aggregate LOD score of 3.21 in the three families. Haplotype sharing within each family was observed for all affected individuals and for obligate carriers in the three families. However, in BU133, three unaffected persons share the complete putative disease haplotype, resulting in negative LOD scores throughout the region (fig. 2). The admixture test as implemented in the program HOMOG was used to test for evidence of locus heterogeneity (Ott 1991). Evidence of linkage to 18q in the presence of heterogeneity was obtained ($\chi^2_{(2)} = 15.16$; $P = .0003$), and the likelihood ratio L2/L0 (1,961:1) approached the suggested criterion of 2,000:1 (Terwilliger and Ott 1994). The conditional probability of linkage was high for BU134 (.99 [.98–1.0]) and for BU233 (.98 [.08–1.00]) and low for BU133 (.43 [.02–1.00]). In the linked families (BU233 and BU134), the multipoint Z_{max} was 3.92. Recombinations in two affected individuals place the disease gene within a 25-cM region, between D18S858 and D18S541 (fig. 3).

A multipoint Z_{max} of 2.44 was obtained at the chromosome 13p region in the three families. The strongest evidence for linkage was observed in BU134 ($Z_{max} = 2.14$ at D13S221), which does not reach the criterion for significance. Follow-up of the 3q region yielded a maximum multipoint LOD score of 1.71, between D3S2398 and D3S1754, with the majority of evidence for linkage observed in BU134 ($Z_{max} = 2.3$ at D3S2455). The admixture test provided weak evidence for linkage in the presence of heterogeneity ($\chi^2_{(2)} = 8.8$; $P = .006$), with odds for linkage and heterogeneity of 83:1. It is unlikely, therefore, that a gene at this location accounts for disease susceptibility in these families.

Discussion

The three families displayed a broad spectrum of severity of phenotype. The most severely affected individuals frequently experience light-headedness and fainting on standing and are unable to perform daily tasks unless treated. In contrast, two mildly affected individuals (5066 and 5068) experience orthostatic light-headedness but are not receiving treatment for the disorder. One individual (5176) is an obligate carrier of the disease gene but experiences no symptoms and has normal BP verified by yearly exams. Uncertainty about the mutation status of apparently unaffected individuals was allowed for in the linkage analysis by the use of a reduced penetrance function. A more conservative approach considers information on haplotype sharing in affected individuals only. The nonparametric-linkage statistic computed by GENEHUNTER is a nonparametric ap-

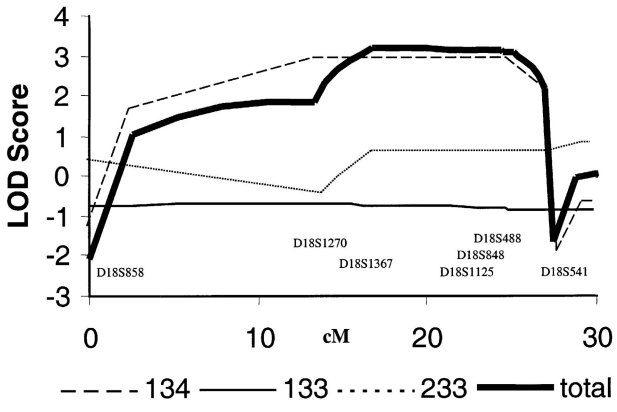

Figure 2 Multipoint linkage maps obtained by the program VITESSE, with the most informative markers on 18q. The markers and their relative distance are shown on the *x*-axis. D18S858 was arbitrarily placed at 0 cM.

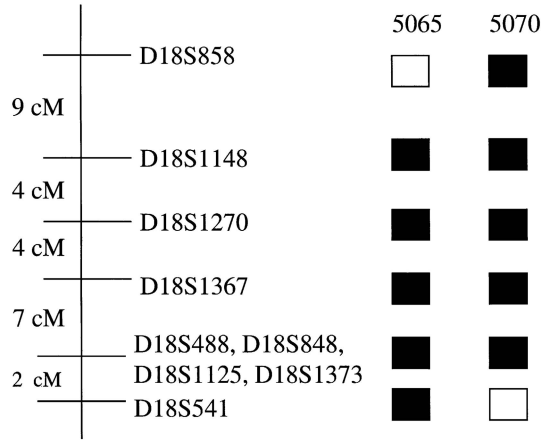

Figure 3 Haplotypes of affected individuals that define the putative disease-gene location for 18q. Blackened squares indicate regions of allele sharing among affected individuals; unblackened squares represent recombinant regions.

proach that evaluates allele sharing among affected relative pairs. In the initial genome screen, evidence for linkage based on the maximum NPL statistic (NPL_{max}) was nearly identical for the three positive regions (3q, $NPL_{max} = 3.95$ [$P = .0078$]; 13p, $NPL_{max} = 4.48$ [$P = .0048$]; and 18q, $NPL_{max} = 4.17$ [$P = .0078$]). The fewest inferred recombinants (unaffected individuals carrying the putative disease haplotype), however, were observed at the chromosome 18 location in the two linked families. Parametric LOD-score analysis with the more liberal penetrance assumption of 80% provided the strongest evidence of linkage to the chromosome 18q location in the two largest families.

Quantitative-trait loci (QTLs) for BP have been mapped to human chromosomes 17q and 8p22 (Wu et al. 1996; Julier et al. 1997), among others. To our knowledge, the chromosomal regions identified in the current study have not been implicated by QTL linkage studies in human populations or in studies using animal models.

There are several serpin (serine protease inhibitor) genes, including those for plasminogen-activator inhibitor-2 (PAI2), maspin, and bomapin, in the chromosome 18 candidate region (Schneider et al. 1995). Although none of these candidate genes have been implicated directly in BP regulation, it remains possible that they possess functions not yet described. Proteases are involved in the formation and degradation of various vasoactive peptides. The inhibition of proteases, through the use of ACE inhibitors, is the basis of one successful therapeutic approach to the control of BP. Therefore, serpins may be an important class of genes for BP regulation, and those located at 18q should be considered as candidates for orthostatic hypotensive disorder. The PAI2 gene was scanned for mutations by use of SSCP. Preliminary examination provided no evidence of mutation in the PAI2 gene in affected individuals in these families.

The original report on these families ruled out known causes of orthostasis and established a common finding of hyperbradykininism, based on high levels of plasma bradykinin and low plasma bradykininase-I concentrations (Streeten et al. 1972). In the quarter century since that report, however, measurements of plasma bradykinin from these patients have given apparently conflicting results, which are actually consistent with the fact that plasma bradykininase concentration can be variable, as described (Streeten et al. 1972). The present study has found no evidence of linkage to regions containing hyperbradykininism-candidate genes, such as the bradykinin receptors B1 and B2 (14q32.1-q32.2) and kallikrein (19q13.2-q13.4). It is interesting to note that the kininogen gene (3q27) is located in the chromosome 3 region that yields a positive LOD score; however, evidence of linkage to this location is weak compared with evidence for linkage to chromosome 18. The possibility that a genetic mechanism for deficient bradykininase synthesis might be responsible for this syndrome is still a tenable hypothesis that remains to be excluded.

Identification of the gene that causes familial orthostatic hypotension disorder will enable genetic testing and could potentially lead to improved treatment for individuals with this condition. Discovery of the mutations in these families, however, may impact more broadly by shedding light on the process of BP regulation. Hypertension, which affects ~20% of the population and is associated with morbidity from stroke, heart attack, and renal disease, can be considered the mirror image of hypotension. This relationship has been proven in the identification of mutations in the epithelial sodium-channel gene, which result in Mendelian forms of both hypotension (pseudohypoaldosteronism type 1; Chang et al. 1996) and hypertension (Liddle syndrome; Hansson et al. 1995). Investigation of candidate genes on 18q may provide additional insights into the role of genetics in BP regulation.

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Electronic-Database Information

Accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for familial orthostatic hypotensive syndrome [MIM 143850])

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