QTL Influencing Blood Pressure Maps to the Region of \textit{PPH1} on Chromosome 2q31-34 in Old Order Amish

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\textbf{Background}—Hypertension is a major risk factor for coronary heart disease, stroke, congestive heart failure, renal insufficiency, and peripheral vascular disease. Although the genetic contribution to variation in blood pressure is well recognized, the specific genes involved are mostly unknown. We carried out a genome-wide scan to identify loci influencing blood pressure in the Old Order Amish population of Lancaster County, Pennsylvania.

\textbf{Methods and Results}—Blood pressures were measured in 694 adult participants from families recruited without regard to blood pressure. We performed a quantitative linkage analysis by using 357 microsatellite markers. In multipoint analysis, strong evidence for linkage was observed with both diastolic (lod = 3.36; \( P = 0.00004 \)) and to a lesser extent systolic (lod = 1.64; \( P = 0.003 \)) blood pressure in the region of chromosome 2q31-34. Peak evidence for linkage occurred at map positions 217 and 221 cM from pter for diastolic and systolic blood pressure, respectively.

\textbf{Conclusions}—A gene linked to familial primary pulmonary hypertension has recently been mapped to this same region, suggesting the intriguing hypothesis that other (attenuated) mutations in this same gene may influence variation in systolic and diastolic blood pressure in this population. \textit{(Circulation. 2000;101:2810-2816.)}

\textbf{Key Words:} blood pressure \textbullet\ Amish \textbullet\ genetics \textbullet\ hypertension, pulmonary

Hypertension is one of the most common chronic diseases in the United States, affecting 20.4\% of adults 18 to 74 years of age and nearly three quarters of African Americans and one half of whites 60 to 74 years of age.\textsuperscript{1} It is a major risk factor for coronary heart disease, stroke, congestive heart failure, end-stage kidney disease, and peripheral vascular disease\textsuperscript{2} and consequently results in tremendous disability and mortality rates.

Variation in blood pressure is influenced by both genetic and environmental factors.\textsuperscript{3,4} Although several rare simple mendelian forms of hypertension have been described,\textsuperscript{3} no underlying cause or transmission pattern can be readily discerned in the vast majority of patients with hypertension. Most family studies indicate that genes account for 20\% to 40\% of the variation in blood pressure levels.\textsuperscript{5–7} To date, however, there has been little progress in identifying the specific genetic defects responsible for the common forms of hypertension.

To identify specific loci influencing variation in blood pressure, we conducted a genome-wide scan in the Old Order Amish (OOA), a genetically isolated white population characterized by large family sizes.

\textbf{Methods}
The OOA population originated in Western Europe (mainly Switzerland), when followers of Jacob Ammann split from their parent Anabaptist sect and emigrated to the United States to escape religious prosecution over a 50-year period beginning in 1727.\textsuperscript{8} Approximately 200 pioneer couples settled in Lancaster County, Pennsylvania, and may be considered founders of the present group of the Lancaster Amish.\textsuperscript{9} The number of OOA in the area is \textgreek{gamma} > 30 000 today,\textsuperscript{10} and nearly all surviving members can be linked to a single 14-generation pedigree.\textsuperscript{11} The OOA are a rural-living population and are characterized by their eschewal of technological innovation and strong interest in their ancestry and genealogical relationships. Furthermore, there is considerable homogeneity in the Amish lifestyle. The majority of men are farmers and the women are mostly homemakers. Fewer than 3\% of the OOA report that they currently smoke, and nearly 90\% of our study participants reported not having any leisure physical activities. Alcohol consumption is minimal. The OOA do not practice birth control, and family members usually eat their meals together.

With the support of the Amish community, recruitment for the Amish Family Diabetes Study began in early 1995 with the goal of identifying susceptibility genes for type 2 diabetes and related traits. The study protocol was approved by the Institutional Review Board at the University of Maryland School of Medicine, and informed consent was obtained from each study participant. With the help of liaisons from the OOA community, we identified individuals with type 2 diabetes. These probands and their family members \( \geq \) 18 years of age were recruited into the study. Between February 1995 and February 1997, 694 subjects received examinations at the Amish Diabetes Research Clinic in Strasburg, Pennsylvania. Appointments were made in advance by home visit, since the Amish do not use telephones or cars. At the clinic, study subjects received an extensive interview regarding their personal medical history and family history.

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of diabetes. Anthropometric measurements and a 3-hour, 75-g oral glucose tolerance test were also performed. Systolic blood pressure (SBP) (1st phase) and diastolic blood pressure (DBP) (5th phase) levels were obtained in duplicate with the use of a standard sphygmomanometer with the patient sitting for ≥5 minutes and were recorded to the nearest 1 mm Hg. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Mean blood pressure and prevalence of hypertension (SBP ≥140 mm Hg or DBP ≥90 mm Hg or current use of antihypertensive medications) were compared between the Amish population and a representative sample of the overall white population in the United States, as assessed by the National Health and Nutrition Examination Survey (NHANES) III, conducted during 1988 to 1991. DNA was extracted from leukocytes, and a screening set of 357 highly polymorphic microsatellite short tandem repeat markers was genotyped from the ABI Prism Linkage Mapping Set (Perkin-Elmer). The mean marker heterozygosity was 0.75, ranging from 0.33 to 0.91. The average interval between markers was 10.2 cM, and the largest gap between markers was 25.4 cM, occurring on chromosome 7.

Quantitative trait linkage analysis was carried out with the use of a variance components methodology, in which we partitioned variation in blood pressure into components attributable to environmental covariates, the additive effects of genes (ie, residual heritability), and a specific quantitative trait locus, or QTL (ie, the linkage component). These analyses were conducted with the use of maximum likelihood procedures as implemented in the SOLAR software package. The additive genetic effect was modeled as a function of the expected genetic covariances between relatives, and the QTL effect was modeled as a function of the identity by descent relationships at the marker locus. The hypothesis of linkage is evaluated by the likelihood ratio test, in which one evaluates whether the locus-specific effect is significantly >0 (ie, H_0: \sigma^2_{QTL}=0 versus H_a: \sigma^2_{QTL}>0). Both multipoint and 2-point linkage analyses were carried out. SBP and DBP were analyzed separately, and in each analysis, we simultaneously adjusted for the effects of sex and sex-specific age and age^2. Individuals currently taking antihypertensive medications (n=33) were excluded from analysis. Thus, the total number of individuals included for linkage analysis was 661.

We derived the distribution of nominal lod scores under the null hypothesis of no linkage empirically by simulation. To generate this distribution, we simulated an unlinked marker locus with 5 equivalent alleles, assigned genotypes to each founder, and then dropped genotypes down through the pedigree based on mendelian expectations and the founder genotypes. The simulated unlinked marker had approximately the same information content (ie, heterozygosity=80%) as the markers used in the genome scan. We then conducted linkage analysis of blood pressures with the simulated unlinked marker. The unlinked marker locus was simulated with the use of PAP4 software, and the linkage analysis on each simulated data set was carried out with the use of the SOLAR software program. We conducted 20 000 replicates and defined the probability of obtaining a false-positive result as the proportion of replicates for which we obtained a specified lod score or higher. These probabilities were then converted into lod scores by first converting them into chi^2 values, and then dividing the chi^2 statistic by (2log10). All lod scores presented in this article were obtained from this simulation.

Although all subjects can be related by tracing their ancestors back multiple generations, to reduce computational difficulties, we divided the sample into 28 discrete families, ranging in size from 3 to 69 individuals. The sample included a large number of relative pairs, including 436 parent-offspring pairs, 1326 sib-pairs, 1342 avuncular (aunt/uncle-niece/nephew) pairs, and 1311 first-cousin pairs.

Results

The mean age of the 694 participating study subjects was 46.5±15.7 years, and mean levels of SBP and DBP were 122.4±17.8 mm Hg and 78.4±9.7 mm Hg, respectively. Mean BMI was higher in women than in men (28.0±5.7 versus 26.2±3.8 kg/m^2). Mean blood pressures for men and women are shown in Figure 1 for both the OOA and the overall US white population. Among men, blood pressures were similar in the OOA compared with the overall US white male population, but OOA women tended to have slightly higher blood pressures than the overall US white female population. In general, the prevalence of hypertension in the OOA women (21.7%) was comparable to that in the US white women, although the prevalence in OOA men (16.1%) is slightly lower than in US white men. The age-specific prevalence rates of hypertension in the OOA are shown in Figure 2. The level of hypertension control in the OOA was low, with only 25% of hypertensive subjects currently under treatment, compared with >50% in the overall US white population.

Heritabilities of SBP and DBP were 0.23±0.07 (P<0.0001) and 0.29±0.07 (P<0.0001), respectively, indicating that a substantial portion of the variation in these traits is attributable to additive genetic factors. Detailed results from our multipoint genome-wide scan are shown in Figure 3. (These results may also be obtained from the SFBR web site at http://www.sfbr.org/sfbr/departments/genetics/genepid/). The maximum lod scores were 3.36 and 1.64 for DBP and

![Figure 1. Sex-specific mean blood pressure levels in OOA and National Health and Nutrition Examination Survey (NHANES) III white population.](image1)

![Figure 2. Sex-specific prevalence of hypertension in OOA.](image2)
SBP, respectively, both occurring in the same region on chromosome 2q, ≈217 to 221 cM from pter. On only 1 other chromosome did we obtain a lod score as high as 1.0 (lod score=1.49 for DBP on the pter end of chromosome 9).

Results from the multipoint linkage analysis for chromosome 2 are shown in Figure 4. Peak evidence for linkage for both SBP and DBP occurred in the region bounded by markers D2S117 and D2S325. The 1-lod unit support interval (ie, the region corresponding to the peak lod score minus 1) for the region encompassing the DBP QTL included a 17-cM interval flanked by markers D2S364 and D2S325, and that for SBP included a 35-cM interval flanked by markers D2S326 and D2S126. The addition of diabetes and BMI as covariates did not substantially alter these results (adjusted lod scores=3.63 and 1.32 for DBP and SBP, respectively, at these same marker positions). There was no evidence for linkage of the dichotomous trait, hypertension, to chromosome 2q markers (data not shown), although the power to detect linkage to the dichotomous trait was low in this sample.

A second region of suggestive linkage to SBP (lod=1.09) was also observed on chromosome 2, ≈56 cM from pter on the short arm. A conditional analysis was performed to determine whether the effect of allele-sharing at this second locus accounted for a significant portion of the trait variation, after accounting for allele-sharing at the locus on chromosome 2q. This hypothesis was evaluated by the likelihood ratio test, in which we compared the likelihood of a 2-locus model (ie, QTL effects at chromosome 2p and 2q) with that of a 1-locus model (QTL effect at chromosome 2q only). The likelihood of the 2-locus (conditional) model was only marginally better than that of the likelihood of the single-locus model, with the marginal lod score of the 2p locus estimated to be only 0.20.

The 2-point linkage analyses provided substantial supporting evidence for linkage of both SBP and DBP to the 20-cM region on chromosome 2q. Three markers were typed within the region of linkage: D2S364 (at 205.2 cM from pter), D2S117 (at 214.6 cM from pter), and D2S325 (at 224.4 cM from pter). The 2-point lod scores associated with these markers were (for DBP and SBP, respectively) 1.41 and 0.81 for D2S364; 3.13 and 1.33 for D2S117; and 1.91 and 1.49 for D2S325.

Discussion

The results from our analyses provide strong evidence for the presence of a gene on the long arm of chromosome 2 that influences variation in systemic blood pressure. This conclusion is bolstered by the fact that linkage was detected in this same region to both DBP and SBP and that consistent evidence for linkage across multiple markers in this region was obtained in the 2-point analyses. The evidence for linkage, as determined by simulation studies, was $P=0.00004$ for DBP and $P=0.003$ for SBP (corresponding to lod scores of 3.36 and 1.64 for DBP and SBP, respectively). We detected virtually no other linkage signals for either trait anywhere else in the genome. Considering that the correlation between SBP and DBP in this population is 0.65, it is possible that a locus on chromosome 2q influences variation in both traits. There are, however, almost certainly additional loci elsewhere in the genome that contribute to variation in either SBP or DBP (or both).

Despite the unique background of the OOA, epidemiological aspects of blood pressure variation in this population appear very similar to the overall US white population. For example, the distribution of blood pressure levels and the prevalence of hypertension in the Amish are comparable to those of the US white population. Furthermore, as in other populations, blood pressure variation in the Amish has a significant familial component, but there is no clear mode of inheritance. Since the Lancaster County OOA arose from ≈200 founding couples who migrated to the United States from Western Europe in the early to mid 1700s, and a subset of the overall US gene pool also originated from this region of Europe, we hypothesize that common gene variants that contribute to blood pressure variation in the Amish are likely to comprise a subset of those that are relevant in the overall US and European white populations. It is possible that the unique characteristics of the OOA population may favor detection of these blood pressure genes since there may be a smaller number of genetic variants segregating in this population, each contributing a greater proportion of variation in blood pressure. In addition, the relatively homogenous lifestyle of the Amish may further make these gene variants easier to detect, since they will account for a larger component of the trait variation. Proof of these hypotheses will require the identification of specific gene variants through positional cloning or positional candidate approaches.

Linkage has previously been reported between hypertension (and/or blood pressure) and several functional candidate genes, including angiotensinogen on chromosome 1q42-43, $\alpha_{1}B$-adrenergic receptor and dopamine receptor type 1A on chromosome 5q31.1-qter, $\alpha_{1}$ lipoprotein lipase on chromosome 8p22, genes encoding the $\beta$ and $\gamma$ subunits of epithelial sodium channel on chromosome 16p12.18 and angiotensin-converting enzyme on chromosome 17q23.19.20 Two recent genome-wide linkage studies using discordant sib-pairs found several linkage signals on 2p21-22.1, 5q33.3-34, 6q23.1-24.1, and 15q25.1-26.13 and regions containing markers D3S2387, D11S2019, D15S657, D16S3396, and D17S1303.22 However, there was no evidence in our analysis for linkage of any of these regions to blood pressure variation. Linkage to blood pressure variation has been reported in several studies with rodent models,23-27 but none of these maps to human chromosome 2.

The fact that we failed to detect linkage to any of these regions in the OOA can be attributed to any of a number of factors, including the possibility that the prior results were false-positives and the lack of power of our study to detect linkages observed in other populations. We estimated the power of our sample to detect QTL effects that accounted for 10% to 30% of the phenotypic variation in blood pressure in our population. These results, obtained by simulation, revealed that we would have 78% power (at lod $\geq$ 3) to detect a QTL that accounted for 30% of the phenotypic variation, 56% power to detect a QTL accounting for 25% of the phenotypic variation, and 33% power to detect a QTL accounting for 20% of the phenotypic variation. Thus, even if these other gene effects did exist, our power to detect them would be low.
Our power to detect linkage to the qualitative trait, hypertension, was far lower, as the number of affected sib-pairs in our sample was 92, 71 (77%) of whom come from only 6 sibships, thus making the number of independent sibships substantially lower.

There are, in addition, substantial differences in study design and analytic strategies between our studies and others that may also account for the failure of our study to detect linkages reported by others. For example, some of the published studies (except for 2 recent discordant pair analyses21,22) did not test for linkage genome-wide but rather evaluated evidence for linkage only to a set of hypertension candidate genes, none of which were on chromosome 2q. Some studies have evaluated evidence for linkage to the dichotomous trait hypertension only,15 whereas others have reported results for SBP only.16,21 Although it is likely that there may be genes with pleiotropic effects on all 3 traits, it is also possible that there are genes whose influence is primarily on 1 of these traits. A further key difference among published studies is the age distribution of the population. Different genes may express their effects at different ages, or their effects may be expressed only in the presence of other

Figure 3.
age-related factors, as, for example, long-term smoking exposure and/or hormonal profile. In our study, the majority of subjects were ≥40 years of age, whereas at least 2 other genome scan studies have focused primarily on younger subjects.21,22 The high proportion of postmenopausal women in our sample may have added an additional source of variability into our study, since different genes may influence blood pressure regulation in the premenopausal and postmenopausal states.

Recently, 2 different groups have independently localized a gene for familial primary pulmonary hypertension (PPH) to a 25-cM region on 2q31-32, an interval corresponding closely to the peak region of linkage in our analysis.28,29 PPH is a rare disease characterized by elevated pulmonary artery pressures in the absence of a secondary cause.30 The disorder leads to right ventricular failure and, in the absence of treatment, death. Young women are at higher risk for the disorder, and an estimated 6% of PPH cases are inherited in an autosomal dominant fashion with reduced penetrance.31 In 1997, Nichols et al28 identified 6 families in which familial PPH was segregating and reported a maximum multipoint lod score of 7.86, occurring at the map position of marker D2S311. On our map, this marker falls within the interval flanked by markers D2S117 and D2S325, the 2 markers that flank the
peak region of linkage in our analysis. In fact, Nichols et al place the location of marker D2S311 at 1.6 cM telomeric to marker D2S117, a position corresponding to position 216.8 cM from pter on the Amish map. Thus, our peak signal for DBP in multipoint analysis occurred at a position <1 cM away from the point of peak linkage reported by Nichols et al, and our peak signal for SBP occurred at a position ∼4 cM away. At about the same time, Morse et al observed significant evidence for linkage (lod = 3.87) with markers in this same region in a single family with autosomal dominant PPH. These investigators mapped the PPH1 locus to a 27-cM region flanked by markers D2S1776 and D2S1384, with peak evidence for linkage also occurring at marker D2S311, but with 6 additional markers (including D2S364) on the centromeric side of D2S311 also providing equally strong evidence for linkage.

To date, PPH1 has not been cloned, nor is its function known. An autoimmune component to the underlying cause of PPH has been suggested on the basis of reported associations between PPH and several autoimmune disorders, and with specific HLA alleles. The candidate region encompassing PPH1 contains other known genes that could influence vascular wall function, such as parathyroid receptor 2 and insulin growth factor binding proteins 2 and 5. In addition, a cluster of immunoglobulin superfamily genes that encode integrin subunits αv, α4, and β6 has been localized to this region.

To our knowledge, there have been no OOA families with PPH. Nevertheless, it is intriguing to speculate that the QTL identified in our analysis of blood pressure variation in the Amish may, in fact, be PPH1, or a linked regulator of this gene. Although PPH is a rare disease (with only 60 families affected family members), it is possible that other defects in this gene may produce a phenotype of systemic blood pressure elevation by affecting systemic endothelial vasculature and/or function.

The results of this genome-wide scan to detect blood pressure genes have revealed the presence of a QTL on chromosome 2q in the OOA that influences DBP and perhaps also SBP. The point of peak linkage coincides closely with the location of PPH1. The identification of this gene, whether it turns out to be PPH1 or a closely linked gene, should enhance our understanding of the cause of hypertension and perhaps lead to novel strategies for the prevention and treatment of this disease.

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