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Use of the Robust Sib-Pair Method to Screen for Single-Locus, Multiple-Locus, and Pleiotropic Effects: Application to Traits Related to Hypertension

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Summary

Robust sib-pair linkage analysis can be used as a screening tool in the search for the potential involvement of single-loci, multiple-loci, and pleiotropic effects of single loci underlying phenotypic variation. Four large families were each ascertained through one adult white male with essential hypertension. The robust sib-pair method was used to screen these families for evidence of linkage between 39 quantitative traits related to hypertension and 25 genetic marker loci. All traits were analyzed on the untransformed, square-root and log-transformed scales. Among other findings, there is a suggestion of linkage between the 6-phosphogluconate dehydrogenase locus on chromosome 1p36 and mean fifth-phase diastolic blood pressure. There may also be linkage between the following markers and traits: the adenylate kinase-1 marker and/or the Lewis blood group marker and the traits height, weight, and biacromial breadth; the glyoxylase I marker and the traits upper-arm circumference and suprailiac skinfold thickness; the ABO blood group and adenylate kinase-1 markers on chromosome 9q34 and the third component of complement marker on chromosome 19p13 and dopamine-β-hydroxylase; and the P1 blood group and the traits weight and 1-h postload serum glucose level.

Introduction

Genetic linkage analysis can be used to identify single-locus effects. The demonstration of genetic linkage between a known polymorphic marker locus and a trait phenotype indicates that the trait has a genetic component involved in its underlying etiology. However, a subtle distinction exists between two different ways linkage analysis can be used to investigate the genetic etiology of "complex" traits (i.e., traits thought to have a genetic component but for which the etiology of that component is, as yet, unknown).

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In the first approach, the phenotype of a trait is assumed to be well identified and usually represents a distinct clinical or biological entity (such as Huntington disease, e.g.). Segregation analysis is used to describe the mode of inheritance of the genetic component and to determine relevant parameter estimates. These estimates are then used in a linkage analysis to find the physical location(s) in the genome relevant to that trait. This is the traditional sequence followed in genetic segregation and linkage studies, and it is most effective when the phenotype is actually determined in large part by segregation at a single Mendelian locus. This approach has been termed *phenometric* analysis (Elston and Wilson 1990), in that it seeks to determine the genotypes underlying specific well-defined phenotypic traits. However, it is a less effective approach when the phenotype is not well defined or is a quantitative trait or when the underlying genetic component of the trait is responsible for a relatively small portion of the phenotypic variation. The second approach as-

sumes that alleles at a putative single locus are involved in the etiology of a clinical or biological entity and that the different genotypes at that locus have some measurable effect on the phenotype. The putative locus is assumed to be well identified through its Mendelian segregation, which can be more precisely measured if data are available at tightly linked marker loci. An attempt is then made to determine the kinds of phenotypic variation, if any, for which its various genotypes are responsible. This second approach, termed *genometric* analysis, seeks to measure the phenotypic actions and interactions of specific genotypes (Elston and Wilson 1990). Use is made of this fact in the Haseman and Elston (1972) robust sib-pair method and in the stepwise oligogenic method for segregation and linkage analysis (Wilson et al. 1990). In the most extreme form of this approach, the marker locus and the putative locus are one and the same, leading to the measured-genotype method of Boerwinkle et al. (1986), or the marker-association method of George and Elston (1987). In the absence of linkage information, pedigree discriminant analysis, another form of genometric analysis, can be used to define the pleiotropic effects of a single locus (Goldin et al. 1980; Zlotnick et al. 1983).

Morton (1990) notes that a linkage test in complex disease is well motivated under either of two conditions: (1) if a prior segregation analysis has given evidence for a major locus or (2) if there is a candidate locus that may influence liability. The latter motivation is inherently different from the former. The notion of a candidate locus—for example, a locus in a biochemical pathway thought to influence the phenotype, without such an effect having been actually demonstrated—is a restricted application of the genometric approach.

In addition to the identification of single loci with major effects, the genometric approach may allow the identification of single loci with relatively minor effects, the identification of oligogenic or multiple loci that affect single traits, and the identification of single loci that have pleiotropic effects (i.e., that affect several phenotypic traits). In the present study we discuss the use of the robust sib-pair method (Haseman and Elston 1972; Elston 1984; Amos 1988, pp. 56–58) as a screening tool to detect possible evidence of linkage, and we apply it to 25 marker loci and 39 quantitative hypertension-related traits in an attempt to identify potential loci that may have single, multiple, or pleiotropic effects on these traits.

Methods

Robust Sib-Pair Linkage Analyses

Although the most powerful sib-pair linkage methods require approximately twice the sample size of lod-score methods (Demenais and Amos 1989), sib-pair methods do not require either any assumption about the mode of inheritance for a particular trait or any knowledge of the parameters specifying the mode of inheritance or age-at-onset distribution for that trait. Sib-pair linkage methods can be broadly classified into those based on the identity-by-state relationships of the sib pairs at the marker locus, such as the Penrose (1953) method, and those based on the sib pair's identity-by-descent (IBD) relationships, such as the method of Haseman and Elston (1972). The essential feature of the Haseman and Elston approach is that information from both the sibs and their parents' marker phenotypes are used to estimate, using Bayesian methods, the proportion of alleles (genes) each sib pair shares IBD at the marker locus. In the case of sibships of size two or larger, it has been shown that a valid test results if all possible sib pairs are included in the analysis as though they were independent (Blackwelder and Elston 1982; Amos et al. 1989).

For quantitative traits, the squared sib-pair trait difference is regressed on the estimated proportion of alleles each sib pair shares IBD at each marker locus. Thus, if a polymorphic marker is tightly linked to a putative locus responsible for at least some of the phenotypic variation of a trait, the alleles at the marker and putative loci should segregate together. Sibs with an estimated proportion of alleles IBD that is high (more likely to be concordant for the marker alleles) should also have a high probability of having the same alleles at the putative linked locus, and the difference between the sibs' phenotypes should be relatively small. Conversely, if the estimated proportion of alleles IBD for the marker locus is low (more likely to be discordant), the probability of having the same alleles at the putative linked locus should be similarly low, and the difference between the sibs' phenotypes should be relatively large. Thus, as the proportion of alleles IBD for the marker locus increases from 0 to 1, the difference between the sibs' trait phenotypes should decrease (i.e., the slope of the regression line should be negative) if a locus tightly linked to the marker is responsible for part of the trait variation. On the other hand, if the marker locus is not linked to a putative locus responsible for some of the phenotypic

variation of the trait, the difference between the phenotypes of the sibs would not be expected to change with the proportion of alleles IBD at the marker locus (i.e., the slope of the regression line would be expected to be 0).

In the present paper, two methods were used to estimate the proportion of alleles IBD for each sib pair. The first method used information from the parents and the sib pairs, while the second method used information from the parents and the entire sibship (Amos 1988, pp. 56–58). Estimating the proportion of alleles IBD by using information from the entire sibship decreases the variance of these estimates, resulting in a more powerful test. It is important to note that most of the information from the robust sib-pair method is derived from pairs of sibs whose estimated proportions of alleles IBD are near 0 or 1. The distribution of these estimates depends, in part, on the number and frequency of the alleles at the marker locus. The number of sib pairs whose estimated proportions of alleles IBD are near 0 or 1 can be small, even if the total number of sib pairs is large, especially if the marker is not particularly polymorphic. In order to ensure that outliers were not responsible for spurious significant results, the analysis for any test significant at a nominal P value $\leq .05$ was repeated after removal of the squared sib-pair differences more than 3 SD from the mean for any given estimated proportion of alleles IBD. Given that the squared sib-pair differences might be expected to be distributed approximately as either a χ^2 or mixture of χ^2's, large squared sib-pair differences were more likely than small differences to be removed as outliers.

Sib-pair analyses were performed on untransformed, square root, and natural log scales for each trait. These transformations were performed to determine the scale on which the underlying genetic component was most additive. Theoretical considerations indicate that an additive scale of measurement maximizes the correlation between the squared sib-pair differences and the proportion of alleles IBD at the linked marker locus. Regressions were performed using both unweighted and weighted least-squares (WLS) regression (Amos et al. 1989). Although the WLS method has been found to be the more powerful on the basis of a limited series of simulations, it was found to be anticonservative when there are fewer than 300 sib pairs (Amos et al. 1989), and nothing is known about its robustness.

Because the sib-pair method is intended as a preliminary screening test, no attempt was made to adjust the traits for age, sex, or other covariate effects. If covariate effects exist, they would decrease the power of the test, but, except as indicated in the discussion, they should neither spuriously increase the sib-pair differences when the estimated proportion of alleles IBD at the marker locus is low nor decrease the sib-pair differences when the estimated proportions of alleles IBD at the marker locus is high.

The results of these analyses can be presented in the form of a marker-locus by trait phenotype matrix (marker-phenotype matrix) with the nominal P values for each marker-phenotype sib-pair test as elements of the matrix. Tentative evidence for genetic linkage can be inferred when a P value for a specific marker-phenotype combination is significant after adjustment for the number of tests performed. If the marker-phenotype matrix is arranged with the marker loci as rows and with the trait phenotypes as columns, tentative evidence for oligogenic or multiple-locus involvement can be inferred when there are multiple significant P values in a single column (i.e., when more than one marker locus demonstrates significant P values with a single trait phenotype). Similarly, tentative evidence for pleiotropic effects of a single locus can be inferred when multiple significant P values occur in a single row (i.e., when a single marker demonstrates significant results with more than one trait phenotype). Pleiotropic effects can be identified in two ways. The first uses a genometric approach; for a given marker locus, we search among all trait phenotypes to identify evidence of linkage between that marker locus and multiple trait phenotypes. The second uses a phenometric approach; here, we select a small set of biologically related trait phenotypes and examine their linkage relationships with a large set of markers to identify a locus or loci that may be linked to all or most of the specified trait phenotypes.

A number of strategies can be used to adjust for the multiple comparisons. A conservative approach would use the Bonferroni method to adjust for the total number of statistical tests, in our case 24 informative markers $\times$ 39 traits $\times$ 3 transformations; however, for an adjusted overall significance level of .01, this would require a nominal significance level of 3.58×10^{-6}. Given that the sib-pair test is intended as a screening tool to detect possible evidence of linkage, a less conservative approach was used. When the test was applied to single traits to identify any marker locus that gives an indication of linkage to that trait, we adjusted only for the number of traits. Similarly, when investigating pleiotropic effects for a given

marker locus, we adjusted only for the number of marker loci.

Application

Hypertension is a major risk factor in the development of cardiovascular disease, a leading cause of death in the United States. A genetic component is thought to be a part of the etiology underlying hypertension, although both the exact mode of inheritance and the nature of the genetic component are unresolved. The evidence supporting a genetic component for hypertension is well documented, being derived primarily from epidemiological studies of general populations, simple family studies including twin and adoption studies, and the estimation of correlations between relatives (Thomas and Cohen 1955; Thomas et al. 1964; Pickering 1968; Vandermolen et al. 1970; Biron et al. 1975; Feinleib et al. 1975; Borhani et al. 1976; Slattery et al. 1988).

Segregation analysis has provided evidence for single-locus effects in studies of several hypertension-related traits, e.g., sodium-lithium countertransport (Dadone et al. 1984; Hasstedt et al. 1988), apolipoprotein AI (Moll et al. 1986), catechol-o-methyltransferase (Siervogel et al. 1984; Wilson et al. 1984), and dopamine-β-hydroxylase (Wilson et al. 1988). However, relatively few segregation analyses of blood pressure have been published. Morton et al. (1980) found no evidence for a major locus for either systolic or diastolic blood pressure. Carter (1984), in a preliminary analysis of systolic blood pressure in the Framingham study, reported that both a single Mendelian locus and an environmental hypothesis were strongly rejected. She concluded that a person's systolic blood pressure is dependent on the parents' systolic blood pressure levels and suggested polygenic or multifactorial transmission as the mode of inheritance. Marazita et al. (1987) found that diastolic blood pressure fitted a mixture of two distributions significantly better than it fitted a single normal distribution, although a single-locus hypothesis was rejected while an environmental hypothesis was not. Rice et al. (1990) found evidence both for two distributions and for a major effect for systolic blood pressure, but they rejected the single-locus Mendelian hypothesis and failed to reject an environmental hypothesis. Rice et al. noted, however, that both the evidence for two distributions in the parental generation (but not in the offspring) and the finding of equal transmission probabilities (nearly equal to 1) are compatible with a hypothesis of a true single-locus effect under the supposition that a recessive genotype for elevated systolic blood pressure has not yet expressed itself in the offspring. Most recently, Myers et al. (1990) have suggested possible evidence for a single recessive locus for systolic blood pressure in nuclear families from the Framingham study.

As part of a genetic investigation of hypertension, we have studied four large families, designated as HGAR 6 and 8–10, each ascertained though one white middle-aged man with diastolic blood pressure >95 mm Hg. The probands were selected from a population originally screened in the National Heart, Lung and Blood Institute's Multiple Risk Factor Intervention Trial but not enrolled in that study. These families, described in detail elsewhere (Siervogel et al. 1980; Siervogel 1983, 1984; Wilson et al. 1984, 1988), comprises 923 individuals more than 8 years old at the time of the study. Twenty-five marker loci were determined on about 600 of these individuals, and various quantitative traits were determined on 500–600 individuals (depending on the trait). The number of sib pairs used in each test depends on the number of pairs with complete data for both the trait and marker locus and varies from 168 to 590 pairs, with at least 500 sib pairs for most of the trait-marker combinations.

In the present analysis, we considered the following 39 quantitative traits: pulse rate; systolic and diastolic blood pressure; height; weight; biacromial and bicristal breadth (shoulder and hip breadth); upper-arm circumference; triceps, subscapular, and suprailiac skinfold thickness; relative weight (weight divided by the median weight for appropriate race, sex, height, and age, with the 25 year olds' median weight being used for those individuals ≥25 years old); 24-h urine volume; creatinine, aldosterone, kallikrein, renin, and dopamine-β-hydroxylase levels (DBH); fasting plasma cholesterol, triglycerides, high- and low-density-lipoprotein cholesterol; 24-h urine sodium and potassium; hemoglobin; hematocrit; white blood count; fasting and 1- and 2-h postload serum glucose levels; blood urea nitrogen; serum uric acid; red blood cell sodium and potassium; serum pH, sodium, potassium, and carbon dioxide; and urine pH.

The 25 markers (with locus symbol and chromosomal location denoted in parentheses) were 6-phosphogluconate dehydrogenase (PGD, 1p36), rhesus blood group (RH, 1p36-p34), phosphoglucomutase (PGM1, 1p22), Duffy blood group (FY, 1q21-22), acid phosphatase (ACP1, 2p25), immunoglobulin κ (IGK, 2p12), transferrin (TF, 3q21), group-specific component (GC, 4q12), MNS blood group (MNS, 4q28-q31), properdin

factor B (BF, 6p21), glyoxylase I (GLO1, 6p21), glutamic pyruvic transaminase (GPT, 8q24), orosomucoid (ORM, 9q34), adenylate kinase-1 (AK1, 9q34), ABO blood group (ABO, 9q34), hemoglobin beta (HBB, 11p15), esterase D (ESD, 13q14), immunoglobulin γ heavy chain (IGHG, 14q32), haptoglobin (HP, 16q22), Kidd blood group (JK, 18q11-q12), third component of complement (C3, 19p13), Lewis blood group LE, 19p13), adenosine deaminase (ADA, 20q13), P blood group (P1, 22q11-qter), and the Kell blood group (KELL, unassigned). Nomenclature follows that of McAlpine et al. (1985). The HBB marker locus was not polymorphic in these families and is therefore not included in the analyses.

Results

There were a total of 936 tests performed on each scale (i.e., 24 informative markers for each of the 39 traits). On average, prior to adjustment for multiple tests, 9.36 of the results on each scale would be significant at the .01 level by chance alone. When sib-pair and parental data were used to estimate the proportion of alleles IBD and when unweighted regression was used to test for a significant regression, results from 42 tests were significant at the .01 level on the untransformed scale, and 38 results were significant on both the square root scale and the log scale. When complete sibship and parental data were used in an unweighted regression analysis, 29 tests were significant at the .01 level on the untransformed scale, and 26 and 23 were significant on the square root scale and log scale, respectively. When complete sibship and parental data were used in a weighted regression analysis, 53, 46, and 42 tests were significant at the .01 level on the untransformed scale, square root scale, and log scale, respectively. This increase in the number of significant results implies either that in this situation the WLS method is more powerful than ordinary linear regression or that the effective sample size is not large enough to ensure that the WLS test is not anticonservative. Because the simulations that focused on the power of the WLS test were limited (Amos et al. 1989) and because the robustness of the WLS test has not been tested, we present the results based on the most conservative of the three methods used: that in which the proportion of alleles IBD was estimated using information from complete sibship and parental data in an unweighted regression.

The marker-phenotype matrix is illustrated in figure 1, with results presented from sib-pair analyses of 24 informative marker loci and 39 quantitative hypertension-related traits under the square root transformation. Chromosomal locations are given to denote groups of linked marker loci. Nominal significance levels have been adjusted for 39 traits by using Bonferroni's method. Similar matrices were obtained for both the untransformed and the log-transformed traits.

Traits that showed a significant negative regression for a particular marker locus both before and after outliers were removed (adjusted P value $\leqslant .05$ or nominal P value $\leqslant .00131$) are presented in table 1, together with the corresponding marker loci. Among other findings, evidence for linkage was found between the ABO blood group locus on chromosome 9q34 and DBH. This linkage has been corroborated by segregation and lod-score linkage analyses (Wilson et al. 1988) and by localization of the structural locus for DBH on 9q34 (Craig et al. 1988). In the earlier lod-score linkage analysis of these same families, the maximum total lod score between the ABO marker locus and DBH (with male and female recombination fractions held equal) was 5.88 at a recombination fraction of .0, under the square root transformation. However, it should be noted that the maximum lod score and corresponding maximum-likelihood estimate of the recombination fraction were dependent on the transformation used (Wilson et al. 1988).

The ABO, AK1, and ORM loci are part of a linkage group located on chromosome 9q34. Distally from the centromere, the order of the loci is thought to be ORM–AK1–ABO, and the estimated recombination fractions are as follows: ORM–AK1, .30; AK1–ABO, .18; and ORM–ABO, .34 (Meera Khan and Smith 1984). In the current study, the sib-pair screening method also suggested linkage to the AK1 locus. These findings are illustrated in figure 1 under the column headed "DBH" and illustrate the utility of the sib-pair method in detecting linkage between closely linked marker loci (ABO–AK1) and a single trait phenotype. However, no linkage between DBH and ORM was indicated. The recombination fraction between ABO and AK1 (.18) is considerably smaller than that between ABO and ORM (.34), and a recombination fraction of the latter magnitude is beyond the power of the method to detect linkage in a sample of this size (Blackwelder and Elston 1982). In addition to the indication of linkage between the linked ABO and AK1 marker loci and DBH, linkage was also suggested with the C3 locus on chromosome 19p13 when outliers were removed. Biochemical and other statistical evidence have suggested that a second locus may also be involved in the variation of the specific activity of DBH (Dunnette and Weinshilboum 1979, 1982; Wilson et al. 1990).

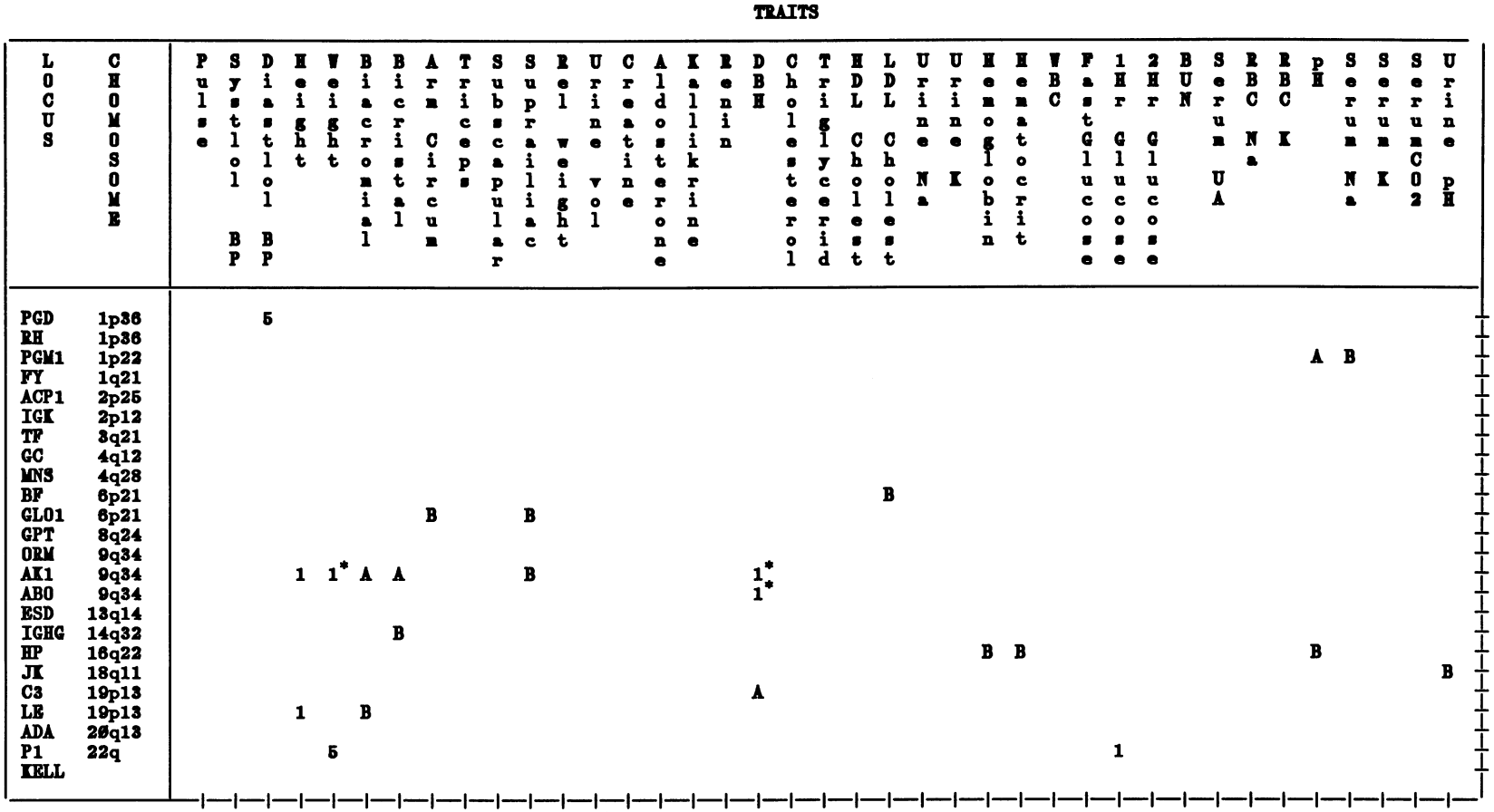

Figure 1 Marker-phenotype matrix from sib-pair analysis of 24 informative marker loci and 39 quantitative traits related to hypertension. Tests with a significant negative regression (adjusted P value $\leq .01$ or nominal P value $\leq .000257$) in the original analysis and in the analysis with outliers removed are denoted by a 1; those with an adjusted significance level of $.01 < P \leq .05$ (nominal P-value $\leq .00131$) are denoted by a 5. Tests both significant in the original analysis at a nominal P value of .00131 and significant at a nominal P value of .000257 after outliers were removed are denoted by 1*. Tests, originally significant at a nominal P value $\leq .05$ but not significant after adjusting for multiple tests, that became significant after outliers were removed at an adjusted P value of .01 or .05 (nominal P values of .000257 and .00131, respectively) are indicated by A and B. See text for a listing of the traits and markers.

Table 1

Traits and Tentative Linked Marker Loci from Sib-Pair Linkage Analysis Screen for Traits Related to Hypertension (nominal *P* values ≤ .00131)

Trait	Scale		
	Untransformed	Square Root	Log
Dopamine-β-hydroxylase	ABO	ABO, AK1	AK1
Diastolic blood pressure	PGD	PGD	
Height	AK1, LE	AK1, LE	AK1, LE
Weight	P1	P1, AK1	AK1, LE
Biacromial breadth			AK1
Upper arm circumference	GL01		
Suprailiac skinfold thickness	GL01		
1-h postload glucose level	P1	P1	P1

Table 1 suggests several other interesting tentative linkages. There is evidence for a possible linkage between PGD on chromosome 1p36 and mean fifth-phase diastolic blood pressure. The *P* values for the regressions of diastolic blood pressure on PGD (.0001, .0004, and .0016 for the untransformed scale, square root scale, and log scale, respectively) were more significant than the corresponding *P* values for the confirmed ABO–DBH linkage (.0003, .0005, and .0348). However, the distribution of the estimates of the proportion of alleles IBD for the PGD marker locus (alleles A and C, with frequencies of .98 and .02, respectively) is relatively sparse. There are 45, 484, and 61 sib pairs whose proportions of alleles IBD are .25, .50, and .75, respectively. The significance levels of the regression of diastolic blood pressure on PGD, when outliers are removed, are .0001, .0005 and .0009 for the untransformed scale, square root scale, and log scale, respectively. The removal of outliers decreases the variance of the estimate of the proportions of alleles IBD, especially at .5. This phenomenon explains the increase in significance often seen when outliers are removed in this sib-pair regression test. Table 1 also suggests tentative evidence of linkage between the following marker loci and traits: the AK1 and/or LE markers and the traits height, weight, and biacromial breadth; the GLO1 marker and the traits upper-arm circumference and suprailiac skinfold thickness; and the P1 blood group and the traits weight and 1-h postload glucose level.

Table 2 illustrates the genometric approach to the identification of pleiotropic effects for the putative locus linked to the PGD marker. Significance levels are presented for any trait with a significant regression on the PGD marker locus (at a nominal significance level ≤.05). If these *P* values are adjusted for the 24 informative marker loci considered, only diastolic blood pressure (on all three scales) remains significant at the .05 level (nominal *P* value = .0021).

Because five of the eight traits indicated in table 1 are anthropometric traits related to body composition, we

Table 2

Traits and Corresponding Nominal Significance Levels for All Indications of Linkage to PGD on at Least One Scale of Measurement (nominal *P* values ≤ .05)

Trait	Scale		
	Untransformed	Square Root	Log
Diastolic blood pressure	.0001	.0004	.0016
Suprailiac skinfold thickness	.0150	.0279	.0409
Total serum cholesterol	.0068	.0178	.0483
LDL cholesterol	.0426		
Serum CO_2	.0080	.0047	.0040
24-h urine volume			.0433
White blood count			.0401[a]

[a] Not significant if outliers are removed.

Table 3

Nominal Significance Levels for Linkage of Anthropometric Measures, under Square Root Transformation, to Any Marker Locus (nominal *P* values ⩽ .05)

	Marker Significance										
Trait	PGD 1p36	ACP1 2p25	IGK 2p12	BF 6p21	GLO1 6p21	ORM 9q34	AK1 9q34	IGHG 14q32	LE 19p13	P1 22q11	KELL
Height			.032				⩽.00005		⩽.00005	.036[a]	
Weight			.037[a]				.001		.017	.0006	
Arm circumference			.037[a]		.003		.042	.048		.004	
Triceps		.017	.032	.029							
Subscapular				.015							
Suprailiac	.028			.014	.004	.032	.014				
Biacromial							.002		.018		.046[a]
Bicristal			.002				.007	.008		.008	
Relative weight					.050					.026	

[a] Not significant if outliers are removed.

also used a phenometric approach in an attempt to identify marker loci that may be involved in the etiology underlying any of the anthropometric traits. Table 3 presents nominal *P* values and the corresponding marker locus for any marker locus that had a significant regression with any of the anthropometric traits (nominal *P* values ⩽.05) on the square root scale. It is interesting to note that six traits suggested linkage with the AK1 marker locus, six with the GLO1 or BF markers on chromosome 6p21, three with the LE marker locus, and five with the P1 marker locus.

Discussion

Robust sib-pair analysis can be used as a screening tool in the search for potential major-locus involvement underlying the phenotypic variation of quantitative traits. When results are presented in the form of a marker-phenotype matrix, in addition to screening for evidence of single-locus effects, the method can be used to identify oligogenic or multiple-locus effects and to identify pleiotropic effects of single loci. Given the confirmation of the ABO–DBH linkage, serious consideration must be given to the other tentative linkages suggested in the present study. Of the 39 quantitative traits considered in the present screening study, the traits identified in table 1 should be the most likely candidates for traditional phenometric segregation and linkage analyses.

However, as with every statistical method, there are situations in which the sib-pair method will produce spurious results. In addition to chance sampling variations, as reflected in the significance level, the method can give false indications of linkage either when there are undetected errors in the pedigree structure (e.g., nonpaternities, nonmaternities, adoptions, or undetected MZ twins) or when the sample exhibits a trait-marker association not due to tight linkage. The latter could occur as a result of either an epistatic effect on viability of two loci, one hitchhiking with a trait locus and the other hitchhiking with the marker locus or genetic heterogeneity in the sample. A marker locus that has a pleiotropic effect on the trait may also lead to a significant result, but this can be formally considered as an effect of linkage—it corresponds to the limiting case of linkage in which there is complete disequilibrium and a recombination fraction of zero.

To address these concerns, precautions were taken to ensure that the data analyzed were consistent with the family structures for all 24 informative markers, known adopted individuals were removed from the families, and like-sex twin pairs with genotyping data available for both twins were examined to determine zygosity. Twins discordant for at least one marker locus were considered to be DZ twins, and both twins were retained in the analyses. In the case in which both like-sex twins were concordant for all marker loci, one twin would be randomly removed, but no like-sex twin pair in these data was concordant at all marker loci.

Trait-marker associations were also investigated. Under the assumption of independence of the trait measures for all individuals, analysis of variance was used to test for significant differences of each trait among marker phenotypes, for those traits and corresponding marker loci presented in table 1. This can be

considered as a numerically simple screening test that usually increases type I but not type II error. After adjustment for multiple comparisons, the only significant difference found at even the 20% significance level was for fifth-phase diastolic blood pressure and the PGD locus, on all three scales (nominal P values $\leq .0008$ and adjusted P values $\leq .01$). On the untransformed scale, the mean fifth-phase diastolic blood pressure was 72.1 for the PGD "AA" phenotype and 79.4 for the PGD "AC" phenotype. This result was then corroborated by allowing for familial correlations (George and Elston 1987); the nominal P value was .005 on the untransformed scale. These findings suggest either that the PGD "C" allele has a pleiotropic effect on fifth-phase diastolic blood pressure (thereby increasing it) or that another locus, tightly linked to and in linkage disequilibrium with the PGD locus, may be responsible for some of the variation of fifth-phase diastolic blood pressure.

It is also interesting to note that Hasstedt et al. (1989) reported evidence for a single major locus for a relative-fat-pattern index derived by dividing subscapular skinfold thickness by the sum of subscapular and suprailiac skinfold thickness. The present study suggests a number of potential linkages that may involve anthropometric measures including weight, biacromial breadth, upper-arm circumference, and suprailiac skinfold thickness.

It should be emphasized that these results were obtained by using a screening methodology and that traditional segregation and parametric linkage analyses of these and other data must be performed to corroborate these findings. In the event that a segregation pattern consistent with that of a single major locus can be demonstrated, a parametric lod-score linkage analysis could then be performed. Similarly, if results from the sib-pair screening test suggest linkage to marker loci in different linkage groups (an oligogenic model), then the method of stepwise oligogenic segregation and linkage analysis (Wilson et al. 1990) can be used in an attempt to identify several major loci involved in the phenotypic variation of the trait. This genometric approach and the measured genotype approach of Boerwinkle et al. (1986) can be used to incorporate information from the linked marker locus, to provide additional information about the underlying segregation of genotypes that may be responsible for the trait. If the sib-pair screening test suggests that a number of traits may be linked to a single marker locus (pleiotropic effects of a single locus), then a multivariate approach would be appropriate. This requires a genetic analysis in the spirit of discriminant analysis, in order to discriminate among the different genotypes at a single locus. Pedigree discriminant functions (Goldin et al. 1980; Zlotnik et al. 1983), linear functions of the traits that are most likely distributed as a mixture of normal distributions and that are segregating in a fashion consistent with that of a single locus, can be determined from a portion of the data and can then be cross-validated on the rest of the data. Bonney et al. (1988) have developed, for a bivariate phenotype, an analogous method that estimates the linear function that maximizes the likelihood of the function being monogenically determined and linked to a marker locus. These and/or other methods will be used in subsequent analyses, in an attempt to identify the underlying putative genetic components.

Clearly, the quantitation of polygenic or multifactorial components of a trait, while of interest academically, can help only minimally in the identification of individuals who have a genetic risk for developing a particular disorder, or in their eventual treatment. The identification of single loci, even if their effects on the phenotype are minor and the identification of their interactions with other genetic loci is a clinically relevant approach in genetic epidemiology. To this end, the robust sib-pair method should prove to be an efficient tool to screen for evidence of linkage between marker loci and trait phenotypes and to detect oligogenic effects, minor single-locus effects, and pleiotropic effects, especially as both the overall number of marker loci available and the number of alleles at each locus increase.

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