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Genome-wide Linkage Analysis of Multiple Metabolic Factors: Evidence of Genetic Heterogeneity

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Abstract

The metabolic syndrome is highly complex disease and has become one of the major public-health challenges worldwide. We sought to identify genetic loci with potential influence on multiple metabolic factors in a white population in Beaver Dam, Wisconsin, and to explore the possibility of genetic heterogeneity by family history of diabetes (FHD). Three metabolic factors were generated using principal component factor analysis, and they represented: *1)* glycemia, *2)* blood pressure, and *3)* combined (body mass index, high-density lipoprotein cholesterol and serum uric acid) factors. Multipoint model-free linkage analysis of these factors with 385 microsatellite marker was performed on 1,055 sib-pairs, using Haseman-Elston regression. Genome-wide suggestive evidence of linkage was found at 30cM on chromosome 22q (empirical P [P_e] = 0.0002) for the glycemia factor, at 188–191 cM on chromosome 1q (P_e = 0.0007) for the blood pressure factor, and at 82 cM on chromosome 17q (P_e = 0.0007) for the combined factor. Subset analyses of the families by FHD showed evidence of genetic heterogeneity, with divergent linkage signals in the subsets on at least 4 chromosomes. We found evidence of genetic heterogeneity by FHD for the three metabolic factors. The results also confirmed findings of previous studies that mapped components of the metabolic syndrome to a chromosome 1q region.

Keywords

linkage mapping; factor analysis; genetic heterogeneity; metabolic syndrome; diabetes mellitus

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DISCLOSURE

The authors have no conflict of interest to declare.

INTRODUCTION

The metabolic syndrome is characterized by clustering of obesity, dyslipidemia, hyperglycemia, and hypertension within individuals. It is highly prevalent and has become one of the major public-health challenges worldwide. According to the third National Health and Nutrition Examination Survey, the prevalence of metabolic syndrome was 24% in US adults (1). This is cause for concern, as adults with metabolic syndrome are atgreater risk for type 2 diabetes (2,3), cardiovascular disease and stroke (4,5). Similarly, we previously found that components of the metabolic syndrome predicted incident cardiovascular diseases and diabetes in an adult population in Beaver Dam, Wisconsin, and persons with a cluster of the components were associated with an increased risk of diabetes, such that those with four or more components were 35 times as likely to develop the disease as those with none of the components (6).

The development of metabolic syndrome clearly involves both environmental factors and largely unknown genetic factors. Data from the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study indicated that individual metabolic traits were highly heritable (e.g. heritability of 46% for body mass index [BMI] and 63% for high-density lipoprotein [HDL] cholesterol), and genetic correlations between individual traits were significant (7). These provide evidence that genetic factors contribute to the manifestations of metabolic syndrome, and also suggest that its genetic mechanisms are complex in nature. It is likely that each individual trait is influenced by several genes, and the genes affecting one trait may also affect some of the other traits (7).

To understand why these metabolic syndrome-related traits cluster in individuals and families, a central question would be whether there is one, or more likely, several genes that influence metabolic traits. To answer this question, investigators have used factor analysis to identify the underlying genetic basis of metabolic syndrome (8–12). Factor analysis can identify the presence of unrelated underlying factors from many inter-correlated traits related to metabolic syndrome. The identified factors may capture the dimensions of the entire syndrome, and each individual factor may be reflective of the “underlying biology”.

In the present study, we attempted *1)* to identify genetic loci with potential influence on multiple metabolic factors, obtained from principal-component factor analysis, and *2)* to investigate genetic heterogeneity of the factors in an adult white population in Beaver Dam, Wisconsin.

METHODS AND PROCEDURES

Study population

The study families were recruited from a population-based study, which was initially designed to determine prevalence, incidence and risk factors for age-related eye disease. Methods used to identify the study participants have been described previously (13). Briefly, a private census of the Beaver Dam population was performed and 5,924 eligible persons aged 43–86 years were identified. From 1988 to 1990, a baseline examination was completed for 4,926 (83.2%) persons, of whom 98.8% were white. Informed consent was obtained from each participant and the study protocol was approved by the Wisconsin-Madison Institutional Review Board.

Based on information collected from the baseline examination, family relationships were identified and extended pedigree information was confirmed during follow-up examinations (14,15). To be a member of a pedigree, an individual needed to be a participant in the baseline examination and be related to another eligible individual through a sibling, parent, child, first cousin or avuncular relationship. There were no inclusions/exclusions based on prior or current evidence of prevalent metabolic or cardiovascular diseases at the time of family ascertainment.

After excluding pedigreesnot informative for linkage (ie, parent-offspring pairs), there were 2,068 people who were members of 486 pedigrees (of 602 original pedigrees), consisting of 1,055 sib-pairs who had complete data on all relevant metabolic traits and have been genotyped for genome-wide microsatellite markers. The mean family size was 10.5 ± 12.5 members.

Metabolic phenotypic data collection

Participants responded to an interview during which they were asked whether they had ever been told by their physicians that they had any of the following: diabetes, angina, a heart attack, a transient ischemic attack, a stroke, and/or hypertension. If participants self-reported diabetes, they were asked if they were currently using insulin, oral hypoglycemia agents, and/or a special diet. Weight and height were measured on a standard scale, with subjects not wearing shoes, and then BMI was calculated. Blood pressure was measured using a random-zero sphygmomanometer following the Hypertension Detection and Follow-up Program protocol (16). Following the examination, casual blood specimens were obtained and serum total cholesterol (17), HDL cholesterol (18), serum glucose, glycosylated hemoglobin (HbA1c) (19), and serum uric acid (20) were assayed according to standard protocols.

Diabetes was defined as either *1)* a previous history of diabetes treated with insulin, oral hypoglycemic agents and/or diet, or without treatment but presence of hyperglycemia at the baseline examination, or *2)* the presence of elevated HbA1c >2 SDs above the mean of the relevant age-sex group at the baseline examination in persons with no previous medical history of diabetes (i.e., newly diagnosed diabetes) (21). Hyperglycemia was defined as HbA1c >2 SDs above the mean of the relevant age-sex group or a casual serum glucose level >200 mg/dl. Primary care providers were consulted whenever there was doubt about the diagnosis.

Principal-component factor analysis

Multiple metabolic factors were derived using factor analysis on seven metabolic traits in the Beaver Dam study population, after adjusting for age, age-squared and gender (age and age squared were included in the regression models because these provided a better fit than age alone). The seven traits were BMI, systolic and diastolic blood pressure, serum glucose, HbA1c, serum HDL cholesterol, and serum uric acid. The principal-components factor method (22) was used to analyze the correlation matrix between these traits. The factors with eigenvalues ≥1 were retained. Data were then rotated using a varimax rotation to generate interpretable components. Factor loadings were examined and loadings ≥0.40 were used to characterize the factor. The scoring coefficients, generated during the factor analysis, were then used to compute factor scores for each individual subject. All principal-component factor analysis was performed using Stata software (version 9.2; Stata Corp., College Station, TX).

Genotyping and genetic analyses

Correlations of the factors obtained from factor analysis between all possible family pairs in the pedigrees were calculated, using the FCOR program of the Statistical Analysis for Genetic Epidemiology (S.A.G.E.) software package (version 5.0). Heritability was estimated for each factor using parent-offspring correlations (r) by the equation $h^2 = 2r$.

A genome-wide scan with 385 microsatellite markers on 22 chromosomes (modified Weber panel 8 marker set) was performed by the Center for Inherited Disease Research (Johns Hopkins University, Baltimore), using automated fluorescent microsatellite analysis. The average spacing of markers was 9 cM throughout the genome and there were no gaps in the map >18 cM. Map distances were based on the Marshfield map (23). Mendelian inconsistencies were checked using MARKERINFO (S.A.G.E) and misspecified family relationships were identified using RELTEST (S.A.G.E.) and RELCHECK (24). Allele frequencies of all the markers were estimated with the FREQ program (S.A.G.E.).

We used the factor scores as continuous traits to localize chromosomal regions responsible for each factor. Prior to linkage analysis, data normality was checked. George-Elston transformation (25) ($\lambda_1 = -1.287$, $\lambda_2 = 0.432$) was performed on factor 1 because of its highly kurtotic distribution. The coefficients of skewness and kurtosis were 0.12 and −0.07 for factor 1 (after transformation), 0.42 and 0.63 for factor 2, 0.18 and 0.23 for factor 3, respectively. These properties of the factor score distributions minimally violated the normality assumption.

Multipoint model-free linkage analyses were performed using Haseman-Elston regression in SIBPAL (S.A.G.E.), which models quantitative sib-pair traits as a function of marker allele identity-by-descent sharing (26). We used a modified Haseman-Elston method, in which the dependent variable is weighted average of the squared trait difference and the squared mean-corrected trait sum, further adjusted for the non-independence of the sibling pairs (option W4 in SIBPAL) (27). This approach has been shown to have more power than the original Haseman-Elston method and its other extensions (27). The nominal P (P_n) values were obtained assuming the asymptotic distribution of the likelihood-ratio test statistics. To verify all multipoint P_n values (<0.01), we performed up to 1,000,000 permutations to obtain empirical P (P_e) values.

In consideration of the potential effects of diabetes and taking hypertension medication on metabolic traits (28–30), Haseman-Elston linkage of each factor was repeated, including diabetes affection status and taking hypertension medication (yes vs. no) as a covariate in the analyses. Furthermore, recognizing that genetic heterogeneity of common complex traits is a thorny issue for linkage analysis, we performed linkage analysis of the factors in two subsets of the families defined by the presence or absence of family history of diabetes (FHD). For a given family, the presence of FHD was defined as at least one member enrolled in the linkage study having been diagnosed with diabetes or having newly diagnosed diabetes at the baseline examination. For a given family, if none of the enrolled family members had diabetes, that family was defined as not having FHD.

RESULTS

Phenotypic characteristics of the Beaver Dam study population and the family members enrolled in the linkage scan are presented in Table 1. The age and sex distribution and the levels of metabolic traits of the family members were similar to those of the entire population. The absolute values of the pairwise correlation coefficients among the seven traits range from 0.756 between HbA1c and serum glucose levels to 0.006 between HbA1c and diastolic blood pressure (Supplementary Table S1). Three factors with eigenvalues ≥1 were identified (Table 2). The first factor correlated highly with serum glucose and HbA1c, and thus was interpreted as a “glycemia” factor. The second factor had high loadings of systolic and diastolic blood pressures, and was labeled as a “blood pressure” factor. The third factor correlated at least moderately with BMI, serum HDL cholesterol and serum uric acid; therefore, it was labeled as a “combined” factor. The three factors together explained 70.9% of the total variance.

Familial correlation among possible relative pairs for each of the three factors is shown in Table 3. Estimates of the heritability were 48% for the glycemia factor, 40% for the combined factor and 17% for the blood pressure factor.

Multipoint linkage analysis of the three factors was performed on the 1,055 sib-pairs. In the Haseman-Elston regression model without covariates (Figure 1a and Table 4), we observed several regions with a multipoint P_n <0.00074, the Lander and Kruglyak’s criterion for suggestive linkage (31), on chromosomes 1, 10, 13, 16, 17, and 22. The glycemia factor had its strongest linkage at 30 cM on chromosome 22 (near maker D22S689). The blood pressure had its strongest linkage signal at 188–191 cM on chromosome 1 (between marker D1S1619

and D1S1589). Other linkage signals for the blood pressure factor with a P_n <0.00074 were observed in three regions on chromosomes 10q, 13q and 16p. The strongest signal for the combined factor was detected at 82 cM on chromosome 17 (marker D17S1290). Overall, the linkage signals of the three individual factors did not cluster together, except that regions linked with the blood pressure factor and the combined factor modestly overlapped on chromosome 1q (Figure 1a). The linkage results that included diabetes (Figure 1b) or taking hypertension medication (data not shown) as a covariate were essentially similar to these models.

A total of 189 persons in these analyses had diabetes at the time of the baseline examination, and 25 (13.2%) of them were newly diagnosed. We stratified families by whether they had FHD. There were 139 families (*n* of sib-pairs = 519) with FHD, and 338 families (*n* of sib-pairs = 531) without FHD; nine families with a member whose diabetes status was missing were excluded from the subset analyses. The mean age (63.5 ± 10.8 years) of members in the families with FHD was not significantly different from that (62.9 ± 10.9 years) in the families without FHD (P = 0.277).

As shown in Table 4 and Figure 2, the families with and without FHD gave different linkage results for the three factors on at least 4 chromosomes. For several regions (ex., chromosome 22q for the glycemia factor, 13q for the blood pressure factor, and 17q for the combined factor), the stratification of families by FHD indicated which subset of families were contributing to the original linkage signal. Moreover, additional linkage peaks were identified after stratification by FHD. For the blood pressure factor, in the families with FHD, a region with genome-wide suggestive evidence of linkage was mapped to 137 cM on chromosome 7 (marker D7S1804, P_n = 0.0004; P_e = 0.0006), which was not detected when all families were analyzed as a whole. Interestingly, chromosome 13 showed a relatively complex pattern of linkage peaks in all families over an extensive distance from 50 cM to 78 cM, which may be actually composed of two peaks derived respectively from the two subsets of families. We observed genome-wide suggestive evidence of linkage for the blood pressure factor to one region at 50 cM on chromosome 13 in the families with FHD (near maker D13S788, P_n = 0.0004; P_e = 0.0007), whereas another region with a stronger evidence of linkage at 78 cM was identified in the families without FHD (near maker D13S793, P_n = 0.00002; P_e = 0.00002). Four of these regions showed significant genetic heterogeneity (P <0.05) between the families with FHD and without FHD (Table 4). Linkage peaks that occurred on or very close to telomeres are reported in Supplementary Table S2. It has been suggested that intervals without flanking markers (as at telomeres) cause an inflation of Type 1 error and thus should be interpreted with caution (32).

To determine whether the evidence of linkage to chromosome 22q for the glycemia factor might reflect linkage to diabetes, we performed additional linkage analysis on diabetes as a discrete phenotype using the SIBPAL program (26). In the 486 families, there were 1,072 sib-pairs with information on both diabetes status and genotypes. The results of the multipoint linkage scan revealed one region with a P_n value <0.00074 (Supplementary Figure S1); it was on chromosome 22q12 (near maker D22S689, P_n = 0.0005; P_e = 0.004). It is noteworthy that this peak was coincident with the peak for glycemia factor in the families with FHD.

DISCUSSION

We used a factor analysis approach with a genome-wide scan to map loci of multiple metabolic factors. Because we only had restricted set of metabolic syndrome traits, we were not aimed to identify genetic loci for metabolic syndrome itself. However, our findings can still be compared to those from studies using an established definition of metabolic syndrome.

One of the strengths of the current study was that the study families were recruited from the general population in Beaver Dam and were not ascertained for any clinical characteristics related to diabetes or metabolic syndrome. Thus, it provided us a unique opportunity to investigate the possibility of genetic heterogeneity. The results did provide evidence for genetic heterogeneity since the two subsets of families defined by FHD were linked to several distinct loci. It has been shown that 45% of first-degree relatives of persons with diabetes were insulin resistant compared with only 20% of individuals without FHD (33). Consistently, individuals with parental history of diabetes or FHD had a 2- to 5-fold higher risk of occurrence of metabolic syndrome (28–30), compared to those without FHD. We assume that genes with likely pleiotropic effects are shared between diabetes and metabolic traits, and yet we are aware that diabetes is likely to be an important effect modifier. Therefore, by accounting for the genetic variance due to family propensity to diabetes, we would have more power to identify loci for the factors underlying metabolic traits. Our study also highlighted the importance of stratification by diabetes status in genetic studies on metabolic traits. Further studies are needed to test whether more precise modeling of family propensity to diabetes would improve the ability to localize causative genes underlying the metabolic factors.

Most linkage scans on metabolic traits have been performed in families which were ascertained for specific clinical characteristics, such as diabetes, obesity, hypertension, or cardiovascular diseases. Using this method of family ascertainment could select pedigrees with a stronger genetic component to some of the metabolic-related traits and thus would improve the ability to localize causative genes. However, the results are limited in generalization. Families which are representative of the general population are of great interest for quantitative traits, and linkage studies on such families should provide less biased results. Interestingly, using such families, the Insulin Resistance Atherosclerosis Study Family Study located a locus for metabolic syndrome to chromosome 1q23-q31 (LOD = 2.6) (34), which is the same region showing evidence of linkage with the blood pressure in the current study.

Factor analysis results are contingent on the number and nature of the traits included and the procedures used in the analysis. Previous studies that have used the principal-component factor analysis approach found 2 to 4 distinct factors representing the metabolic syndrome (8–12, 35). However, none of them, including the present study, has been able to replicate each others' findings. Nevertheless, the chromosome 1q24.3 region found in our study appears to confirm previous linkage findings. This finding is interesting that 1q region, which is essentially identified as type 2 diabetes region (36), failed to show any evidence of linkage with glycemia factor, the factor that accounts for the largest amount of the variability, but showed correspondence with blood pressure factor. Subsequent linkage scans on metabolic syndrome and related quantitative traits, have also detected linkage in the same region but across a broader area of chromosome 1q (10,34). Collectively, the present and previous studies strongly suggest chromosome 1q may harbor a gene which has pleiotropic effects, and more likely, several genes which have individual or additive effects on components of the metabolic syndrome. This is further supported by the findings that the lamin A/C gene, located on chromosome 1q21, was shown to be associated with the development of dyslipidemia and metabolic syndrome (37).

In the present study, the second highest peak for the blood pressure factor was identified on chromosome 16p12.3-16p12.1. This region contains *SCNN1B* gene, which is a causative gene for Liddle syndrome, a mendelian form of hypertension (38). Common genetic variants in *SCNN1B* were shown to affect blood pressure in the general population (39).

We included serum uric acid as a metabolic trait in our analysis. We and others have previously shown that serum uric acid is predictive of incident diabetes and cardiovascular disease (6, 40), and is also an independent determinant of metabolic syndrome (41). In the current study,

we found serum uric acid loaded with BMI and HDL cholesterol, which supports the use of serum uric acid as a metabolic syndrome-related trait. The phenotypic correlations among BMI, HDL cholesterol and serum uric acid in the current study (0.23–0.32 in absolute value, Supplementary Table S1) were actually very close to those estimated from the NHLBI Family Heart Study (0.25–0.36 in absolute value), in which significant genetic correlations were also found between serum uric acid and BMI or HDL cholesterol (7).

We note limitations to the current study. First, the major limitation of the present study is the phenotype itself. Our data on metabolic traits were limited due to the study being designed initially as a study of age-related eye disease. However, although the initial focus of the study was eye diseases, the study families were not ascertained based on any eye disease, but rather were a representative sample from the general population, so this should not bias the results of our study. Second, the serum glucose level measured in this study was not fasting. The influence of a non-fasting glucose in the analysis is that addition of variance in serum glucose level due to daily dietary intake would make environmental factors stronger, and thus decrease our study's power to detect genetic factors. However, this would render results presented here conservative, without an increase in Type I error. Additionally, the use of composite scores of HbA1c and serum glucose from factor analysis, rather than individual traits, would help to reduce the effect of measurement errors. Lastly, we defined FHD as any family member with diabetes. Some of the families are quite extended, with the possibility that in a family with FHD, a portion of the family members are not either first or second degree relatives to individuals with diabetes. This may contribute to increased genetic heterogeneity which would be expected to decrease power but not increase Type I error.

In summary, with the use of the families ascertained from a homogeneous population in Beaver Dam, we found substantial evidence of genetic heterogeneity by family propensity to diabetes for multiple metabolic factors. The multiple distinct loci found across the genome also suggest complex pathogenesis of the metabolic factors. In addition, our results reconfirm the findings of previous studies which mapped components of metabolic syndrome to the chromosome 1q region.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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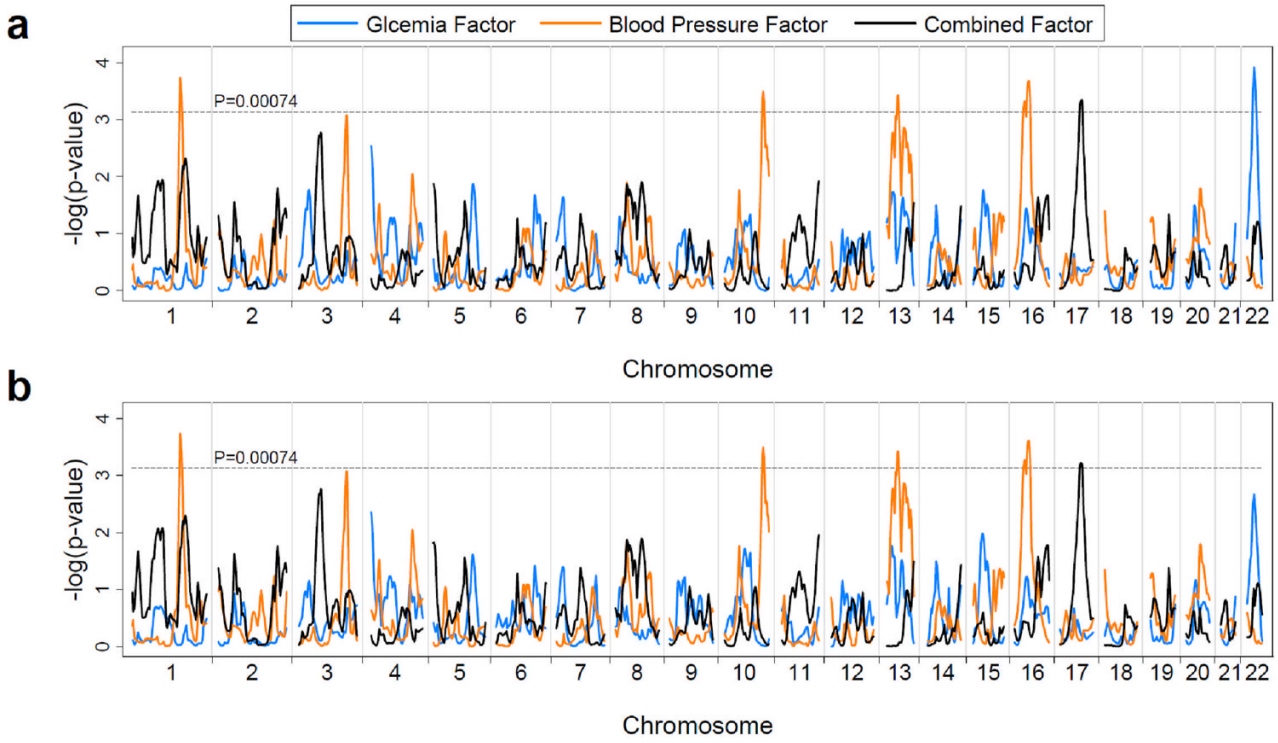

Figure 1.

Genome-wide multipoint linkage analyses for the three metabolic factors: nominal *P* values of Haseman-Elston regression without **(a)** and with **(b)** diabetes as a covariate.

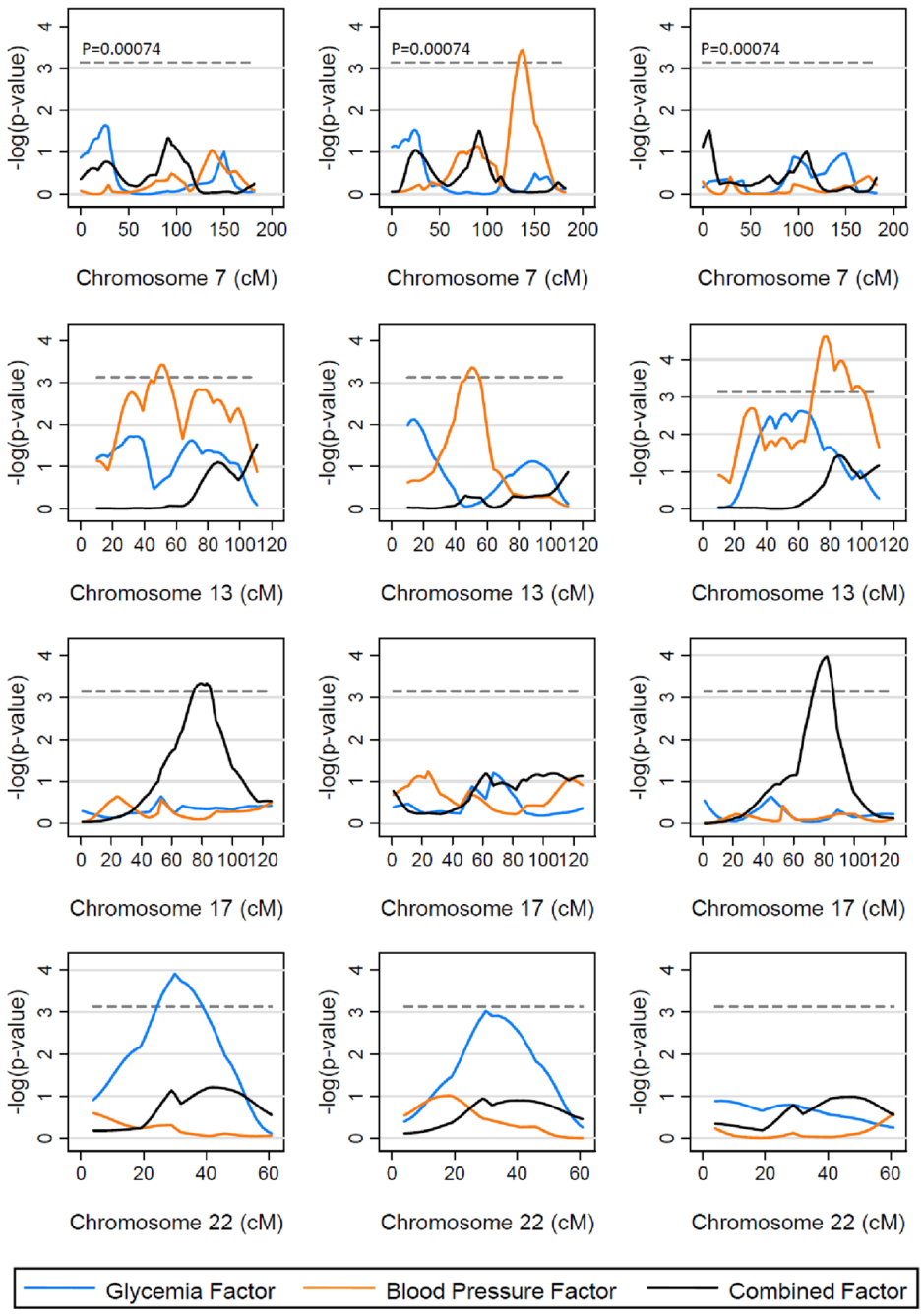

Figure 2.
Nominal P values of linkage analyses for the three metabolic factors on four chromosomes in all families (n of sib-pairs = 1,055; the left column), the subset of families with history of diabetes (n of sib-pairs = 519; the middle column), and the subset of families without history of diabetes (n of sib-pairs = 531, the right column).

Table 1

Characteristics of the Beaver Dam study population and the family members enrolled in the linkage study

Characteristics	Entire study population (*n* = 4,926)	Members of families (*n* = 2,068)
Age (years)	62.0 ± 11.2	63.1 ± 10.9
Gender (% female)	56.1	54.7
Diabetes (%)	9.1	9.2
Serum glucose (mg/dl)	106.7 ± 39.4	107.1 ± 39.7
HbA1c (%)	6.1 ± 1.6	6.1 ± 1.6
Serum HDL cholesterol (mg/dl)	52.0 ± 17.6	51.4 ± 17.3
Serum uric acid (mg/dl)	6.0 ± 1.9	6.0 ± 1.8
BMI (kg/m^2)	28.8 ± 5.4	29.1 ± 5.6
Systolic blood pressure (mmHg)	132.1 ± 20.5	133.8 ± 20.8
Diastolic blood pressure (mmHg)	77.3 ± 11.0	77.6 ± 11.1

HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; BMI, body mass index.

Data are reported as means ± SD, except for gender and diabetes, which are expressed as percentage of subjects.

Table 2

Factor loadings of principal component analysis after varimax rotation

Traits[a]	Factor 1 (glycemia)	Factor 2 (blood pressure)	Factor 3 (combined)
Serum glucose	**0.93**	0.04	−0.04
HbA1c	**0.93**	0.01	−0.05
Serum HDL cholesterol	−0.18	0.13	**0.70**
Serum uric acid	−0.10	0.09	**−0.74**
BMI	0.17	0.27	**−0.68**
Systolic blood pressure	0.09	**0.88**	−0.06
Diastolic blood pressure	−0.03	**0.89**	−0.04
Eigenvalue	2.09	1.61	1.26
Total variance (%)	29.8	23.0	18.1
Accumulate variance (%)	29.8	52.8	70.9

HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; BMI, body mass index.

Data in bold denote absolute values of loadings ≥0.4.

[a] Adjusted for age, age-squared and gender.

Table 3

Familial correlations of the three metabolic factors

		Glycemia factor	Blood pressure factor	Combined factor
Relationship	***n***	**Correlation ± S.E.**	**Correlation ± S.E.**	**Correlation ± S.E.**
Full Sibling	1,061	0.074 ± 0.034	0.120 ± 0.035	0.207 ± 0.036
Parent-offspring	368	0.240 ± 0.053	0.085 ± 0.056	0.199 ± 0.056
Half-sibling	61	−0.002 ± 0.133	0.193 ± 0.130	0.194 ± 0.134
Avuncular	686	0.174 ± 0.041	0.020 ± 0.045	0.036 ± 0.047
Half-avuncular	9	0.405 ± 0.302	0.763 ± 0.163	−0.276 ± 0.352
Cousin	1,559	−0.010 ± 0.027	0.062 ± 0.031	−0.021 ± 0.030
Half-cousin	10	−0.000 ± 0.212	−0.004 ± 0.253	−0.022 ± 0.323

See Table 2 for the constructs of the three factors.

Table 4

Genome-wide linkage results for the three metabolic factors with a multipoint nominal *P* value <0.00074[a]

			All families (*n* of sib-pairs = 1,055)		Families with history of diabetes (*n* of sib-pairs = 519)		Families without history of diabetes (*n* of sib-pairs = 531)		
Cytogenic location	**Position (cM)[b]**	**Closest maker**	**Nominal *P* value**	**Empirical *P* value**	**Nominal *P* value**	**Empirical *P* value**	**Nominal *P* value**	**Empirical *P* value**	***P* for heterogeneity[c]**
Glycemia factor									
22q12.3	30	D22S689	0.0001[a]	0.0002[a]	0.0009	0.001	0.166	–	0.029
Blood pressure factor									
1q24.3	188–191	D1S1619, D1S1589	0.0002[a]	0.0007[a]	0.007	0.009	0.008	0.022	0.907
7q32.3	137	D7S1804	0.089	–	0.0004[a]	0.0006[a]	0.776	–	0.004
10q26.13-10q26.3	149–166	D10S1656, D10S1248	0.0003[a]	0.001	0.006	0.008	0.003	0.012	0.636
13q21.32	50	D13S788	0.0004[a]	0.001	0.0004[a]	0.0007[a]	0.012	–	0.753
13q32.2	78	D13S793	0.001	0.003	0.129	–	0.00002[a]	0.0002[a]	0.003
16p12.3-16p12.1	32–56	D16S3103, D16S540	0.0002[a]	0.0006[a]	0.009	0.010	0.001	0.004	0.555
Combined factor									
17q23.2	82	D17S1290	0.0005[a]	0.0007[a]	0.147	–	0.0001[a]	0.0001[a]	0.048

See Table 2 for the constructs of the three factors.

"–" indicates the empirical *P* values were not obtained because of their nominal *P* values ≥0.01.

[a] *P* <0.00074, the Lander and Kruglyak's criteria for genome-wide suggestive linkage.

[b] Map distances were based on the Marshfield map.

[c] By Z test for difference between the two Haseman-Elston regression coefficients from the two subsets of families.