

Analysis of Metabolic Syndrome Phenotypes in Framingham Heart Study Families From Genetic Analysis Workshop 13

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Twelve teams of investigators constituted a group which analyzed phenotypes related to metabolic syndrome, making use of the available longitudinal measurements from the family component of the Framingham Heart Study or the simulated data, as distributed by Genetic Analysis Workshop 13 (GAW13). Body mass index, obesity, lipid abnormalities, glucose, or combinations of these traits were analyzed by this group. A wide variety of approaches were taken to construct phenotypes from the longitudinal measurements, including considering single or multiple cross-sectional time points, single ages, minimum values, maximum values, means, other lifetime values, ever/never dichotomy, or age at onset of some threshold value. Approaches also differed in the family structures utilized (sib pairs to full extended pedigrees), the genetic data considered (two-point or multipoint), and the statistics calculated (model-free and parametric), and led to a diverse set of analyses being performed. Inferences were made about heritability, and attempts were made to map underlying genes. Over 40 genome-wide linkage analyses were conducted. Despite the broad range of approaches, several regions of the genome were repeatedly identified across multiple analyses. *Genet Epidemiol* 25 (Suppl. 1):S78–S89, 2003. Published 2003 Wiley-Liss, Inc.†

Key words: metabolic syndrome; longitudinal measures; linkage methods; susceptibility genes

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INTRODUCTION

Often obesity, dyslipidemia, hypertension, and diabetes cluster together, a pattern known as metabolic syndrome, syndrome X, insulin resistance syndrome, or the deadly quartet. Medically, this syndrome is important, as it is characterized by an increased risk for cardiovascular disease and type 2 diabetes [DeFronzo and Ferrannini, 1991; Kaplan, 1989; Lakka et al., 2002; National Cholesterol Education Program, 2002; Reaven, 1988]. Components of metabolic syndrome are abdominal obesity, atherogenic dyslipidemia (AD) (elevated plasma triglyceride, small low-density lipoprotein (LDL) particles, and low levels of high-density lipoproteins (HDL cholesterol)),

insulin resistance (with or without glucose intolerance), elevated blood pressure, a proinflammatory state, and a prothrombotic state, with ethnic variability [Abbasi et al., 2002; Araneta et al., 2002; Ferrannini et al., 1997; Saad et al., 1991].

Despite the abundant epidemiologic and experimental research that has been published on metabolic syndrome, definitions of this syndrome vary widely [Laaksonen et al., 2002; Liese et al., 1998]. The National Cholesterol Education Program Expert Panel (NCEP) [NCEP, 2002] recently published criteria for a clinical diagnosis of metabolic syndrome requiring three or more of the following: fasting plasma glucose of at least 110 mg/dl (6.1 mmol/l), serum triglycerides of at least 150 mg/dl (1.7 mmol/l), serum HDL

cholesterol less than 40 mg/dl (1.04 mmol/l) and 50 mg/dl (1.30 mmol/l) for men and women, respectively, blood pressure of at least 130 mm Hg systolic or 85 mm Hg diastolic, or waist girth (a measure of central adiposity) of more than 102 cm for males and more than 88 cm for females.

Although there is clear evidence of common underlying factors in metabolic syndrome, most genetic epidemiological studies have investigated the underlying phenotypes separately. A handful of genome-wide screen linkage studies examining traits individually support the existence of common genetic influences on multiple components of this syndrome [Atwood et al., 2002; Hong et al., 1997, 1998; Kissebah et al., 2000; Liese et al., 1997]. Moreover, a few studies explicitly attempted to dissect the multivariate nature of metabolic syndrome, and found evidence of common genetic factors [Arya et al., 2003; Mitchell et al., 1996]. The GAW13 data set offers a unique opportunity to examine components of metabolic syndrome from multiple perspectives, because data were available longitudinally from multiple phenotypes related to this syndrome. The 12 papers to be summarized here all analyzed some aspect of metabolic syndrome. In this summary, we will describe the methods, results, and discussion of the papers from this group of investigators.

METHODS

CONSTRUCTION OF PHENOTYPES

A major challenge of GAW13 was the enormous volume and complexity of the data, which included multiple different measurements made repeatedly, but sometimes differently, across two generations. From the data provided, the contributors in this group used a variety of approaches to construct phenotypes, in some cases based on a single lipid-related or body mass phenotype, and in others using a broader metabolic syndrome phenotype. Some groups selected a single particularly informative time point [Martin et al., 2003; Stein et al., 2003] or age [Moslehi et al., 2003] on clinical grounds, while others compared the results at multiple time points [Lee et al., 2003; North et al., 2003]. Some groups constructed lifetime measures in a variety of ways. For quantitative phenotypes, one approach was to focus on the maximum [Allen-Brady et al., 2003] or minimum [Horne et al., 2003] value, while others considered instead the average value over time [Geller et al., 2003; McQueen et al.,

2003]. For binary phenotypes, one group defined a trait to be considered present if it was *ever* observed [Yip et al., 2003]. Another group used modeling to construct the phenotype as part of a broader new methodological approach [Ghosh et al., 2003]. One group focused on age at onset, using in part a survival approach [Engelman et al., 2003]. It should be noted that one other critical approach, that of repeated measures, was not included in Group 10, but is covered elsewhere [Gauderman et al., 2003].

LINKAGE AND ASSOCIATION METHODS

The unifying theme of the 12 sets of investigators comprising GAW13 Group 10 was the analysis of a phenotype or phenotypes that related to metabolic syndrome, but the approaches used to test linkage and association were diverse.

Standard applications. For the more standard approaches, two-point and multipoint, parametric and nonparametric methods, and sib pairs to extended pedigree structures were represented. Exact two-point quantitative and qualitative parametric linkage analyses were performed on full pedigree structures as implemented in LINKAGE [Lathrop et al., 1986] by Horne et al. [2003], and using a modified version of VITESSE [O'Connell and Weeks, 1995] by Yip et al. [2003]. Exact multipoint quantitative parametric linkage analysis was carried out using GENEHUNTER [Kong and Cox, 1997; Kruglyak et al., 1996] on pedigrees of restricted size [Horne et al., 2003].

Several different methods were used for calculation of nonparametric linkage statistics. Affected sib-pair analysis using exact identity by descent (IBD) calculations for dichotomous traits was implemented with GAS [Young, 1993] by Yip et al. [2003]. A variety of Haseman-Elston regression-based variants for exact likelihood linkage analysis for quantitative and qualitative traits were performed on sibships using SAGE [SAGE, 2002; Engelman et al., 2003; Moslehi et al., 2003; Yip et al., 2003]. Nonparametric statistics were also applied to pedigrees of restricted size. Exact multipoint, model-free, pedigree regression-based linkage analysis, as implemented in MERLIN [Abecasis et al., 2002], was applied by Geller et al. [2003]. Exact multipoint standard nonparametric linkage analyses in GENEHUNTER were also performed by Yip et al. [2003].

Variance components methods were used by some investigators [Geller et al., 2003; Horne et al., 2003; Martin et al., 2003; McQueen et al., 2003;

North et al., 2003]. They carried out two-point and estimated multipoint variance-component linkage analyses on the full pedigree structures as implemented by SOLAR [Almasy and Blangero, 1998]. Bivariate variance components analyses were performed by Martin et al. [2003], using SOLAR. Exact multipoint variance-component linkage analyses were also performed in MERLIN on restricted pedigree structures [Geller et al., 2003].

Novel methodologies and applications. Several papers introduced novel methodologies (or used existing methods in some novel way) for linkage or association analysis. Ghosh et al. [2003] introduced a novel extension to a linkage method that utilizes the contrast function in sibships in a robust regression-based linkage statistic [Ghosh and Reich, 2002]. This new adaptation for longitudinal data uses sibship data and standardizes over the multiple time points considered. The method assesses linkage significance empirically, and is thus robust to underlying genetic model distributions. This new method was tested among the 100 replicates for the simulated total cholesterol quantitative phenotype in sibships from the offspring cohort.

Some existing methodologies were reintroduced in analyses of Framingham data. Horne et al. [2003] used a Markov chain Monte Carlo blocked Gibbs sampling method, as implemented in MCLINK [Thomas et al., 2000], to estimate multipoint inheritance vectors in extended pedigrees. These were used to calculate the multipoint theta-LOD (TLOD), a hybrid linkage statistic which uses multipoint inheritance vectors at a marker position in a two-point linkage statistic. This method boasts the advantages of using both multipoint analysis, with respect to inheritance information, and two-point analysis, with respect to increased robustness to model misspecification [Göring and Terwilliger, 2000; Abkevich et al., 2001]. In addition, Horne et al. [2003] used by-pedigree linkage scores that were extracted from MCLINK for additional comparisons across analyses at the pedigree level. These by-pedigree scores were proposed as a promising tool to evaluate potential linkage regions.

Two different approaches were taken to reduce multivariate data. Stein et al. [2003] performed exact multipoint, multivariate linkage analysis with full sibling pairs, using structural equation modeling (SEM) in a likelihood ratio framework to simultaneously analyze five component traits of metabolic syndrome. The original concept was introduced by Eaves et al. [1996], and is imple-

mented in the program Mx [Neale, 1999]. The basic underlying method is to fit two models, one with the SEM alone and one with the SEM and the IBD probabilities for each sibling pair at the locus of interest. Linkage to that locus is then tested using a likelihood ratio test. This technique provides a new, truly multivariate method for locating genes involved in syndromes such as metabolic syndrome, and additionally should increase power to locate genes with pleiotropic effects. A different approach was taken by Martin et al. [2003], who attempted to reduce metabolic syndrome multivariate traits by applying factor analyses to the genotypic and phenotypic correlation matrices, and then went a step further by computing factors from a pairwise correlation matrix of genome-wide LOD scores. In this way, they could determine how metabolic syndrome symptoms cluster according to different components of variance.

A novel association-based analysis was introduced by Allen-Brady et al. [2003]. They used an intuitively simple empirical approach to extend case-control association analysis to extended pedigrees. The benefit of such a method is the ability to utilize already ascertained linkage resources for valid association analyses. This method was applied in the simulated data, which were determined to have only low, and unintended, linkage disequilibrium between adjacent microsatellite markers. Microsatellite markers are not ideal for association tests, and it will be interesting to see this method implemented with single-nucleotide polymorphism data elsewhere.

RESULTS

SINGLE TIME-POINT APPROACHES

Three separate contributions constructed metabolic syndrome phenotypes from the Framingham data by selecting information from a single time point. Two of these papers [Martin et al., 2003; Stein et al., 2003] utilized a single examination and examined the suite of metabolic syndrome traits using multivariate statistics. The other paper [Moslehi et al., 2003] attempted to identify QTLs influencing body mass index (BMI) at a single age.

With respect to the single examination, Stein et al. [2003] addressed the question of identifying QTLs for metabolic syndrome. The impetus for this paper was that there is a known shared environmental influence on the components of metabolic syndrome, which if unaccounted for, may inflate the genetic variance. As described

above, they searched the genome for QTLs, using structural equation models (SEM) to covariate adjusted measurements taken at the fifth time point in the offspring cohort for systolic blood pressure (SBP), *ln* fasting plasma glucose (for which an additional power transform was applied), *ln* triglycerides, HDL cholesterol, and BMI. They chose the final time point, hypothesizing that study subjects would best demonstrate any progression to metabolic syndrome by this time point. For the genome scan, two SEMs were evaluated for each sib-pair, i.e., a model with only cross-trait covariances, and a model with cross-trait covariances weighted by allele-sharing IBD at a locus. Their results indicated that the QTLs were mainly influenced by glucose, BMI, and SBP.

Martin et al. [2003] addressed the question of how to reduce multivariate data. The impetus for this paper was that in common complex traits,

genes are likely to exert an effect on multiple traits. Previously, factor analysis was used to generate the factors from the phenotypic and genetic correlation matrix but not from genome-wide LOD score correlations. Therefore, they used bivariate variance components analysis in SOLAR to estimate the phenotypic and genetic correlations between cholesterol, HDL, triglycerides, systolic blood pressure, and BMI. Additionally, they ran variance-components linkage analysis at 10-cM intervals for these traits, and estimated the correlations between LOD scores for each pair of traits. The correlation matrices were factor-analyzed, using SAS with varimax rotation. They reported a pattern of loadings that were different across matrices, suggesting that we may gain more information about the interactions of traits by using multiple approaches.

TABLE I. LOD signals >3.0 with supporting evidence^a

	Signal	Location (cM)	1-LOD interval	Phenotype	Method	Software
Chromosome 2						
North et al.	3.4	151	129–158	HDL exam 11	MPT-VC	SOLAR
Martin et al.	2.6	150		HDL	MPT-VC	SOLAR
North et al.	1.1	122		HDL exam 20	MPT-VC	SOLAR
Moslehi et al.	1.0	167		BMI	MPT-sib-pair-nonpar	SAGE
McQueen et al.	1.7	179		Glucose	MPT-VC	SOLAR
Stein et al.	1.6	180.6		MS	MPT-sib-pair-nonpar	Mx
Chromosome 11						
Moslehi et al.	3.0	143.1	134–end	BMI	MPT-sib-pair-nonpar	SAGE
Engelman et al.	1.4	134.1		Overweight	2PT-sib-pair-nonpar	SAGE
Horne et al.	1.6	161.7		Low TG:HDL	2PT-par	LINKAGE
Horne et al.	1.1	161.7		Low TG:HDL	MPT-MCMC-par	MCLINK
<i>Strug et al.</i>	2.1	131		Mean gain BMI	2PT-VC	SOLAR
Chromosome 16						
Geller et al.	3.2	76	52–83	BMI	MPT-VC	SOLAR
Geller et al.	2.8	78.6		BMI	MPT-VC	MERLIN
Geller et al.	2.5	63.7		BMI	MPT-Reg	MERLIN
McQueen et al.	1.3	75		BMI	MPT-VC	SOLAR
Engelman et al.	1.2	63.7		Overweight survival	2PT-sib-pair-nonpar	SAGE
<i>Li et al.</i>	2.9	46		<i>Mean BMI(4 time points)</i>	<i>MPT-VC</i>	<i>SOLAR</i>
<i>Cheng et al.</i>	~1.1	~70		<i>Mean BMI (3 exams)</i>	<i>MPT-VC</i>	<i>SOLAR</i>
Chromosome 19						
Moslehi et al.	3.3	86.4	NA (2PT)	BMI	2PT-sib-pair-nonpar	SAGE
Moslehi et al.	1.8	86.4		BMI	MPT-sib-pair-nonpar	SAGE
Engelman et al.	1.0	86.4		Obese-survival	2PT-sib-pair-nonpar	SAGE
Martin et al.	1.0	80		HDL	MPT-VC	SOLAR
Chromosome 22						
Horne et al.	3.4	20.9	NA (2PT)	Low TG:HDL	2PT-par	LINKAGE
North et al.	1.4	19		HDL exam 15	MPT-VC	SOLAR
Horne et al.	1.3	20.9		Low TG:HDL	2PT-VC	SOLAR
Horne et al.	1.0	20.9		Low TG:HDL	MPT-MCMC-par	MCLINK

^aEntries shown in italics are from other groups. Locations are in Haldane map units. MS, metabolic syndrome; MPT, multipoint; 2PT, two-point; VC, variance-components; Reg, regression; nonpar, nonparametric; par, parametric.

Moslehi et al. [2003] addressed the question of identifying QTLs for BMI at a single age. They chose to analyze the first BMI value available for each individual measured between ages 40–50 from the parental and offspring cohort, hypothesizing that BMI at this age should be a stable measurement. Significant familial correlation estimated in the SAGE program FCOR justifies the utilization of this age range. Two-point sib-pair regression analysis was performed using SAGE SIBPAL. The strongest evidence for linkage from two-point analysis was at D19S246 ($P=0.000051$) on chromosome 19. A string of markers on chromosomes 2, 3, and 11 gave suggestive evidence for linkage, using multipoint analysis. The regions on chromosomes 2 and 3 are consistent with other published data [Deng et al., 2002; Wu et al., 2002]. Modest evidence for linkage was also found on chromosome 6 ($P=0.03$), overlapping the region identified by Atwood et al. [2002] in this same data set.

MULTIPLE TIME-POINT APPROACHES

Two separate contributions constructed metabolic syndrome phenotypes from the longitudinal Framingham data by selecting information from multiple time points. North et al. [2003] examined HDL in females at three time points, and Lee et al. [2003] examined the correlations of multiple metabolic syndrome phenotypes (glucose, total cholesterol, HDL, SBP, and BMI) at multiple time points.

North et al. [2003] explored the evidence for linkage of HDL at three time points (t_1 , t_2 , and t_3), spaced approximately 8 years apart and corresponding respectively to visits 11, 15, and 20 for the parental cohort, and visits 1, 2, and 4 for the offspring and spouses. Using variance-component methods implemented in SOLAR, they estimated the heritability and genetic correlation of HDL at each time point, performed linkage analysis of HDL at each time point in males and females, separately and combined, and tested for genotype \times sex interaction at a QTL at each time point. North et al. [2003] found significant and suggestive evidence for a QTL on chromosome 2q influencing HDL variation in females across time points (LOD at $t_1=3.2$, $t_2=1.9$, and $t_3=2.4$). These results are similar to those of Almasy et al. [1999], who reported a linkage of unesterified HDL to chromosome 2q at 140 cM, but did not report an interaction with sex.

Lee et al. [2003] examined the familial aggregation of components of metabolic syndrome (glucose, total cholesterol, HDL, SBP, and BMI) across multiple time points in both the parental cohort and offspring cohort groups combined. The parental cohort and offspring cohort examinations were combined, using the calendar date of examinations, into two groups from 1971–1975 and 1984–1987. For the parental cohort, two examinations were averaged for each time point. Sibling-sibling, parent-offspring, avuncular, cousin, and spousal correlations were calculated on age- and sex-adjusted residuals from SAS, using FCOR in SAGE 4.1. Lee et al. [2003] found that the correlations between pairs of relatives were consistent with a pattern of genetic inheritance and across the two time points examined for all the components of metabolic syndrome examined. However, they found that spousal correlations were higher than expected under the genetic model. Using a commingling analysis implemented in SEGREG in SAGE 4.1, the three-mean model fit best for each of the variables examined, supporting a pattern of genetic inheritance.

LIFETIME APPROACHES

Several authors used the longitudinal observations available to measure some lifetime aspect of metabolic syndrome or its components. A lifetime quantitative phenotype using longitudinal data can be defined in different ways, such as an extreme observation (maximum or minimum) or an average. A dichotomous phenotype can be created from a quantitative trait in a longitudinal data set, according to one or more thresholds at one or more time points.

Three contributions addressed the genetics of atherogenic dyslipidemia (AD). Using the Framingham data, Horne et al. [2003] performed linkage analysis on the minimum of the ratio of serum triglyceride (TG) to HDL, while Yip et al. [2003] chose to manipulate the serum TG and HDL levels to produce a dichotomous trait. Allen-Brady et al. [2003] tested for association of candidate regions in the simulated data for maximum HDL. No contributors chose to create a phenotype that sought to address the three lipid abnormalities that characterize AD (TG, HDL, and small low-density lipoprotein particles). Yip et al. [2003] classified an individual as affected with AD if serum HDL was at or below the 25th percentile for age and sex, and serum TG was at or above the

90th percentile for age and sex. An individual was classified as unaffected if serum HDL was above the 25th percentile for age and sex, and serum TG was below the 90th percentile for age and sex. To avoid a trichotomy, Yip et al. [2003] folded the individuals with high TG only or low HDL only into an *unknown* category.

With the minimum TG:HDL ratio adjusted for age, BMI, SBP, and blood sugar (BS), Horne et al. [2003] were able to obtain a two-point LOD score over 3.0 with LINKAGE (see Table I). However, Yip et al. [2003] were not able to find a LOD score ≥ 1.0 with their AD dichotomous phenotype for the same region. This result is somewhat puzzling, given the similarity of the phenotypic definitions of these two teams of investigators. From a purely statistical perspective, one expects a continuous random variable to carry with it more information than a categorical variable, and thus to be more powerful. On the other hand, the careful application of thresholds when categorizing a continuous variable can be successful in linkage analyses by eliminating near-phenocopies or capturing a single gene of major effect. The choices of thresholds by Yip et al. [2003] for serum TG at the 90th percentile and HDL at the 25th percentile are reasonable. However, the classification of individuals (over 500) with high TG only or low HDL only into the *unknown* category may have decreased the amount of linkage information in the sample. Additionally, differences in pedigree structures used in each study may have played a role.

Allen-Brady et al. [2003] performed association analyses using full extended pedigrees considering five simulated candidate regions for a dichotomous trait, such that cases and controls were classified as the upper and lower quartile extremes of maximum lifetime HDL, respectively. Valid tests for association were established using an empirical approach, which is robust to the familial correlations inherent in pedigrees [Camp and Farnham, 2001]. Results indicated the importance of correcting for familial correlations in association tests, and highlighted the lack of power in simply choosing microsatellite linkage markers for association studies. On analyzing underlying linkage disequilibrium (LD) in the five candidate gene regions, there was only one gene region for which significant LD existed in sufficient replicates to study. In these replicates, LD was significant but low; however, the levels of LD were comparable to those observed for the real GAW13 Framingham data for markers with the

same resolution. This is not surprising, since LD was not purposefully simulated and thus not intended, and the simulations attempted to parallel the real GAW13 Framingham data. This study also suggests that the use of microsatellite marker data for association testing should be done with prudence.

The contribution by McQueen et al. [2003] was unique among those performing a lifetime analysis in choosing to study metabolic syndrome. They did so by defining a quantitative composite phenotype derived from TG, HDL, SBP, BS, and BMI. The variables SBP, HDL, and BMI were judged approximately normally distributed. However, TG was log-transformed to obtain approximate normality, and BS was ranked across all measurements and then the resulting ranks were normalized. Each of these variables was regressed on the number of cigarettes smoked per day and four categorical variables representing alcohol consumption, and then the residuals were standardized within groups defined by gender and 1-year age bands. A metabolic syndrome score (MSS) was defined as a linear combination of the five standardized residuals ($MSS = SBP - HDL + TG + BS + BMI$). Finally, these sums were averaged to obtain a lifetime MSS. Multipoint linkage analysis did not reveal any LOD scores of 3.0 or more. Multipoint analyses were conducted with the standardized residuals of each of the individual traits SBP, HDL, TG, BS, and BMI, but no significant linkage was detected.

One genome screen [Geller et al., 2003] examined body mass using a lifetime definition. They used a log-transform of average BMI adjusted by regression for age and smoking (cigarettes per day) for each sex and cohort separately, and found a LOD score over 3.0 (see Table I). Evidence was also found on chromosome 6, with a LOD score of 2.7. Geller et al. [2003] also compared the results of SOLAR with those obtained from MERLIN-VC and MERLIN-REGRESS. The peaks were generally consistent among the three methods, but there were some differences in significance levels.

Analyses using data at all available time points in the lifetime of an individual are expected to be more informative and hence more powerful from an inference point of view, compared with a summary measure based on a representative time point or a simple average of all time points. Ghosh et al. [2003] defined a measure standardized by the variance-covariance matrix of the observations over the time points. They considered the total cholesterol level in the younger cohort of the

simulated data set. For each sibship, they defined a contrast function as a linear combination of the trait (total cholesterol) values with sum of the coefficients equal to 0. Ghosh and Reich [2002] had developed a linear regression of the squared contrast function on a quadratic function of the matrix of IBD scores of different sib pairs within a sibship, but had found that the regression does not perform well as the level of dominance increases. As an alternative, Ghosh et al. [2003] proposed a nonparametric regression based on kernel-smoothing [Silverman, 1986], using the two variables. Since the answers were available to them, they evaluated the power of their method by the proportion of replications in which a significant linkage peak was within a 10-cM window of the true position of a QTL. For 4 of the 6 simulated genes controlling total cholesterol levels, they found significant evidence of linkage in more than 30% of the replications.

AGE-AT-ONSET APPROACHES

Studies show that complex diseases often have a variable age at onset, and there may be substantial loss of power in linkage and/or association analyses which ignore the dependence on age. Engelman et al. [2003] considered two different linkage analyses accounting for age at onset of the phenotypes defined as obesity and overweight in the Framingham data. Their first analysis was restricted to individuals with an age at onset prior to 35 years; they classified an individual as overweight or obese if his or her BMI was greater than 27 or 30, respectively. The paradigm of this restricted analysis was to differentiate between susceptibility genes for early and late ages at onset. Their second analysis aimed at testing linkage to the variation of age at onset of obesity/overweight throughout the lifespan by defining the phenotypes BMI, overweight, and obesity as "residual" from a survival analysis perspective [Commenges, 1994; Hanson and Knowler, 1998]. Both analyses were performed on sib-pairs. The strongest linkage result was obtained on chromosome 1 for obesity in the restricted analysis, but did not replicate in the survival analysis residual method, indicating possible differentiation in susceptibility genes before and after age 35 years. Evidence for linkage on chromosomes 5 and 7 was found by both methods. The two methods did not provide consistent linkage results for overweight, which might be due to a strong environmental compo-

nent controlling weight after the age at onset of 35 considered in the analysis.

DISCUSSION

SUMMARY OF LINKAGE REGIONS IDENTIFIED IN THE GAW13 FRAMINGHAM DATA

In this group of papers, more than 40 genome-wide linkage analyses for a variety of phenotypes relating to metabolic syndrome in the Framingham sample were performed using several different methodologies and computer software applications. A variety of two-point and multipoint, parametric and nonparametric statistics were calculated in these studies, and family structures from sib-pairs to full extended pedigree structures were utilized. Despite the diversity of approaches and phenotypes selected by each participant in the group, several consistent regions were identified. Table I illustrates regions which were identified by a single group with a signal equivalent to a LOD score of at least 3.0. Table II shows consistent regions, defined such that at least one analysis identified a signal over 2.0, and at least two further analyses found evidence of a LOD score ≥ 1 .

Analyses that considered larger pedigree structures identified 9 of the 14 regions listed in Tables I and II. Two regions were equally well-identified by methods incorporating large pedigrees and methods using sib-pair data only. For the remaining 3 regions, sib-pair analyses were superior. This illustrates that there is often more power to detect linkage when analyzing multigenerational pedigrees compared to breaking the same sample into nuclear families or sib-pairs. This observation is consistent with several earlier findings from GAW and elsewhere [e.g., Duggirala et al., 1997].

Five regions were identified with signals of LOD score ≥ 3.0 . These were on chromosome 2 (multipoint 3.4 at 151 cM) [North et al., 2003], chromosome 11 (two-point 3.0 at 143.1 cM) [Moslehi et al., 2003], chromosome 16 (multipoint 3.2 at 76 cM) [Geller et al., 2003], chromosome 19 (two-point LOD score=3.3 at 86.4 cM) [Moslehi et al., 2003], and chromosome 22 (two-point LOD score=3.4 at 20.9 cM) [Horne et al., 2003]. For each of these regions, supportive signals from other genome-wide searches with signals of LOD score ≥ 1.0 were noted (Table I).

For the region on chromosome 2 (multipoint LOD score=3.4), five additional analyses were supportive, with LOD evidence ranging from

TABLE II. Consistent Regions: at least one signal with LOD >2.0 plus additional support from other studies with LOD >1.0^a

	Signal	Location (cM)	1-LOD interval	Phenotype	Method	Software
Chromosome 1						
Engelman et al.	2.3	302	NA (2PT)	Obese	2PT-sib-pair-nonpar	SAGE
Horne et al.	1.9	280.1		High TG:HDL	MPT-MCMC-par	MCLINK
Engelman et al.	1.3	294.4		Overweight-survival	2PT-sib-pair-nonpar	SAGE
North et al.	1.2	297		HDL exam 11	MPT-VC	SOLAR
Martin et al.	1.1	290		HDL	MPT-VC	SOLAR
Horne et al.	1.0	280.1		High TG:HDL	2PT-par	LINKAGE
<i>Strug et al.</i>	2.2	164		<i>Mean gain BMI</i>	2PT-VC	SOLAR
<i>Strug et al.</i>	2.1	164		<i>Mean BMI</i>	2PT-VC	SOLAR
Chromosome 3						
Moslehi et al.	2.1	177.1	160–220	BMI	MPT-sib-pair-nonpar	SAGE
Moslehi et al.	2.0	200.2		BMI	2PT-sib-pair-nonpar	SAGE
Horne et al.	2.0	194.5		High TG:HDL	MPT-MCMC-par	MCLINK
Horne et al.	1.9	194.5		High TG:HDL	2PT-par	LINKAGE
Horne et al.	1.5	181		TG:HDL	MPT-VC	SOLAR
Horne et al.	1.4	181		TG:HDL	2PT-VC	SOLAR
McQueen et al.	1.3	168		Glucose	MPT-VC	SOLAR
Horne et al.	1.1	166.9		High TG:HDL	MPT-par	GH
<i>Cheng et al.</i>	~1.5	~165		<i>Mean BMI (3 exams)</i>	MPT-VC	SOLAR
Chromosome 5						
Horne et al.	2.6	125.2	73–132	High TG:HDL	MPT-par	GH
Horne et al.	1.6	125.2		High TG:HDL	MPT-MCMC-par	MCLINK
Geller et al.	1.5	100		BMI	MPT-VC	SOLAR
Geller et al.	1.3	99.5		BMI	MPT-VC	MERLIN
Geller et al.	1.1	87.8		BMI	MPT-Reg	MERLIN
Engelman et al.	1.1	125.2		Obese	2PT-sib-pair-nonpar	SAGE
Chromosome 6						
Geller et al.	2.7	150.4	141–164	BMI	MPT-VC	MERLIN
Geller et al.	2.1	150.4		BMI	MPT-Reg	MERLIN
Geller et al.	1.9	156		BMI	MPT-VC	SOLAR
Martin et al.	1.7	160		BMI	MPT-VC	SOLAR
Yip et al.	1.4	159.3		AD	2PT-par	VITESSE
Martin et al.	1.4	150		HDL	MPT-VC	SOLAR
Horne et al.	1.3	159.3		High TG:HDL	MPT-MCMC-par	MCLINK
Martin et al.	1.3	150		ln (TG)	MPT-VC	SOLAR
McQueen et al.	1.3	170		MS	MPT-VC	SOLAR
Horne et al.	1.2	150.4		TG:HDL	2PT-VC	SOLAR
Horne et al.	1.1	150		TG:HDL	MPT-VC	SOLAR
Horne et al.	1.1	149.3		High TG:HDL	MPT-par	GH
North et al.	1.0	150		HDL exam 11	MPT-VC	SOLAR
<i>Liu et al.</i>	2.5	146		<i>BMI</i>	2PT-VC	SOLAR
<i>Liu et al.</i>	2.4	155		<i>BMI</i>	2PT-VC	SOLAR
<i>Arya et al.</i>	3.9	158		<i>ln BMI</i>	<i>MPT-univariate</i>	SOLAR
<i>Arya et al.</i>	2.7	150		<i>ln HDL-C</i>	<i>MPT-univariate</i>	SOLAR
<i>Arya et al.</i>	6.2	152		<i>ln BMI-ln HDL-C</i>	<i>MPT-bivariate</i>	SOLAR
<i>Cheng et al.</i>	~1.6	~139		<i>Mean BMI (3 exams)</i>	MPT-VC	SOLAR
Chromosome 7a						
McQueen et al.	2.1	54	36–66	HDL	MPT-VC	SOLAR
Martin et al.	2.0	50		Chol	MPT-VC	SOLAR
Horne et al.	2.0	51.5		High TG:HDL	MPT-par	GH
Chromosome 7b						
Horne et al.	2.7	163.4	NA (2PT)	TG:HDL	2PT-VC	SOLAR
Horne et al.	2.1	170	155–178	TG:HDL	MPT-VC	SOLAR
North et al.	1.6	157		HDL exam 15	MPT-VC	SOLAR
Horne et al.	1.5	158.9		High TG:HDL	MPT-par	GH

TABLE II. Continued

	Signal	Location (cM)	1-LOD interval	Phenotype	Method	Software
Martin et al.	1.4	170		ln (TG)	MPT-VC	SOLAR
Horne et al.	1.2	163.4		High TG:HDL	MPT-MCMC-par	MCLINK
North et al.	1.1	177		HDL exam 20	MPT-VC	SOLAR
Chromosome 10						
Engelman et al.	2.6	4.7	NA (2PT)	Overweight-survival	2PT-sib-pair-nonpar	SAGE
Moslehi et al.	1.7	4.7		BMI	MPT-sib-pair-nonpar	SAGE
North et al.	1.3	22		HDL exam 15	MPT-VC	SOLAR
<i>Liu et al.</i>	1.4	46		<i>BMI-gluc-SBP factor</i>	2PT-VC	SOLAR
Chromosome 14						
Yip et al.	2.0	0	0-30	AD	MPT-Kong and Cox NPL	GH
Horne et al.	1.7	18.5		High TG:DHL	MPT-par	GH
North et al.	1.4	3		HDL exam 20	MPT-VC	SOLAR
Engelman et al.	1.2	28.6		Overweight	2PT-sib-pair-nonpar	SAGE
North et al.	1.0	7		HDL exam 15	MPT-VC	SOLAR
Chromosome 17						
Horne et al.	2.1	129	110-end	Low TG:HDL	MPT-par	GH
Horne et al.	1.5	131.1		Low TG:HDL	MPT-MCMC-par	MCLINK
Yip et al.	1.2	127.5		AD	2PT-ASP	GAS
Horne et al.	1.2	138		Low TG:HDL	2PT-par	LINKAGE
Horne et al.	1.1	127.5		TG:HDL	2PT-VC	SOLAR

^aEntries shown in italics are from other groups. Locations are in Haldane map units. MS, metabolic syndrome; AD, atherogenic dyslipidemia; MPT, multipoint; 2PT, two-point; VC, variance-components; Reg, regression; nonpar, nonparametric; par, parametric.

1.0–2.6, and all were multipoint and from model-free analyses. The phenotypes, however, were diverse, including HDL, BMI, glucose, and a derived metabolic syndrome phenotype using structural equation modeling [Stein et al., 2003]. This region may be a good candidate for a gene involved in metabolic syndrome, rather than any particular single trait, since this region is implicated for many phenotypes of metabolic syndrome and a derived metabolic syndrome phenotype.

The finding on chromosome 11 (equivalent to multipoint LOD score=3.0) was for BMI. Three additional analyses were supportive in this region: one for another BMI phenotype (overweight, BMI >27 kg/m²), but the remaining two for a low TG:HDL ratio. The signal for the overweight phenotype [Engelman et al., 2003], however, was at 134.1 cM, and was much closer to that of Moslehi et al. [2003], compared with those for a low TG:HDL ratio [Horne et al., 2003], which were more telomeric (161.7 cM) and may indicate a different region. If this is the case, this region may be more likely involved in body mass, rather than the complete syndrome.

The region on chromosome 16 (multipoint LOD score=3.2) was for a linkage analysis of BMI. Four additional analyses were supportive for this

region, with nonparametric LOD scores ranging from 1.2–2.8. Three of the supportive findings were for analyses using BMI phenotypes, and the fourth for an overweight phenotype based on a survival analysis. Thus, this region shows strong evidence for a locus containing a gene implicated in body mass.

Similarly, chromosome 19 (two-point LOD score=3.3) was identified in a genome-wide analysis for genes involved in BMI. Additional supportive evidence for this region was found in three analyses: a multipoint analysis of the same BMI phenotype, an obesity phenotype based on survival, and an HDL phenotype. All analyses were nonparametric.

The finding on chromosome 22 (two-point LOD 3.4) was found for the low TG:HDL phenotype. Three additional analyses were supportive, including both parametric and nonparametric linkage analyses. All findings related to lipid phenotypes and thus this region may therefore be implicated in lipid levels, rather than the broader metabolic syndrome.

Nine consistent regions were found, each defined as a region where at least one signal equivalent to a LOD score ≥ 2.0 was found, and two other distinct analyses indicated the same region with a LOD score at least ≥ 1.0 (Table II). Of

these regions, two regions on chromosome 5 (six analyses with LOD scores ≥ 1.0 , best multipoint LOD score=2.6) and chromosome 6 (13 analyses with LOD scores ≥ 1.0 , best multipoint LOD score=2.7) had the highest multipoint LOD support and stood out on inspection as areas of high consistency. Both these regions were identified by a diverse set of analysis types.

Six linkage signals to a region on chromosome 5 at 100–125 cM were found, and 5 of the 6 signals were for multipoint analyses on extended pedigrees (variance-components and parametric linkage analysis). The best signal (LOD score=2.6) was for a high TG:HDL ratio. Other phenotypes that scored in this region were BMI and obesity.

The region on chromosome 6 around 150–170 cM was outstanding, with 13 linkage analyses signaling over LOD 1.0. Eleven of the 13 signals were multipoint. Both parametric and nonparametric (variance-components) methods identified the region, with the unifying factor that all used extended pedigrees. The best four signals were for BMI phenotypes; however, many other phenotypes also implicated this region: AD [Yip et al., 2003]; HDL [Martin et al., 2003; North et al., 2003]; natural log of triglycerides [Martin et al., 2003]; TG:HDL ratio [Horne et al., 2003]; and a derived metabolic syndrome phenotype [McQueen et al., 2003]. Notwithstanding the lack of a LOD score over 3.0, this region may be the best candidate region found in the collective studies of this group for a gene involved in metabolic syndrome.

COMPARISONS TO FINDINGS FROM OTHER GROUPS

Since over half of the contributors to this group analyzed BMI or some dichotomy based on BMI, we thought it was important to compare our results with respect to whole-genome scans for BMI loci to the findings of investigators in other GAW13 groups. Investigators in other GAW groups used a variety of approaches to construct BMI phenotypes. The majority of these groups looked at mean BMI over either all measurements or over a number of time points [Arya et al., 2003; Cheng et al., 2003; Li et al., 2003; Strug et al., 2003]. One group constructed multivariate factors that included BMI [Liu et al., 2003].

Several investigators identified BMI loci on five chromosomes with LOD scores ≥ 3 (Table I). Four investigators from other GAW groups reported LOD scores of >1 for BMI in the regions our group identified with LOD scores ≥ 3.0 (Table I).

Three of these four investigators reported LOD scores of >1.0 , with markers in regions that overlapped with the regions reported by this group. Strug et al. [2003] reported a LOD score of 2.1 for BMI with markers at around 131 cM of chromosome 11. Li et al. [2003] and Cheng et al. [2003] reported LOD scores of 2.9 and ~ 1.1 , respectively, for BMI with markers within the same linked region on chromosome 16 identified by some of the Group 10 investigators.

The consistent regions identified in Table II also had supportive evidence from reports by investigators from other GAW13 groups. Cheng et al. [2003] reported a LOD score of ~ 1.5 for BMI, with markers at ~ 165 cM of chromosome 3 within the region reported by several Group 10 investigators as containing a locus linked to BMI. Of particular note was the locus on chromosome 6 at 150.4 cM (1-LOD interval, 141–164 cM), where three investigators from other GAW groups also found substantial linkage evidence. Liu et al. [2003] reported LOD scores of 2.5 and 2.4 with markers on this region of chromosome 6 with BMI at 146 cM and 155 cM, respectively. Cheng et al. [2003] also reported a LOD score of ~ 1.6 with markers on this region. Arya et al. [2003] reported a LOD score of 3.9 between markers on chromosome 6 at 158 cM and BMI. Arya et al. [2003] also reported a LOD score of 6.2 between markers on chromosome 6 at 152 cM and a BMI-HDL factor. The findings by Arya et al. [2003] strengthen the evidence presented earlier in this paper that this region of chromosome 6 may contain a gene involved in metabolic syndrome.

CONCLUSIONS

The Framingham Heart Study is unique in scope, and allows for genetic analyses of traits having a large impact on public health. The longitudinal data collection allows many unique questions to be addressed. All the participants of this group analyzed one or more components of metabolic syndrome, but vastly different approaches were taken in defining phenotypes over the life span, including analyses at single ages, single or multiple cross sections, minimum or maximum, average, ever/never dichotomy, lifetime, and age at onset. All of these questions are biologically meaningful. A number of novel methods and novel ways of applying existing methods were presented. While many different findings were obtained based on the approaches

taken, there was also converging evidence among many analyses. Several regions of the genome appear to be promising in terms of containing susceptibility genes for one or more traits, and provide a basis for future studies.

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