Original Article

Coincident Linkage of Type 2 Diabetes, Metabolic Syndrome, and Measures of Cardiovascular Disease in a Genome Scan of the Diabetes Heart Study

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Cardiovascular disease (CVD) is a major contributor to morbidity and mortality in type 2 diabetes, but the relationship between CVD and type 2 diabetes is not well understood. The Diabetes Heart Study is a study of type 2 diabetes–enriched families extensively phenotyped for measures of CVD, type 2 diabetes, and metabolic syndrome. A total of 977 Caucasian subjects from 358 pedigrees (575 type 2 diabetic relative pairs) with at least two individuals with type 2 diabetes and, where possible, unaffected siblings were included in a genome scan. Qualitative traits evaluated in this analysis are with or without the presence of coronary calcified plaque (CCP) and with or without carotid calcified plaque (CarCP) measured by electrocardiogram–gated helical computed tomography. In addition, prevalent CVD was measured using two definitions: CVD1, based on self-reported history of clinical CVD (393 subjects), and CVD2, defined as CVD1 and/or CCP >400 (606 subjects). These discrete traits (type 2 diabetes, metabolic syndrome, CVD1, CVD2, CCP, and CarCP) frequently coincide in the same individuals with concordance ranging from 42.9 to 99%. Multipoint nonparametric linkage analysis revealed evidence for coincident mapping of each trait (type 2 diabetes, metabolic syndrome, CVD1, CVD2, CCP, and CarCP) to three different genomic regions: a broad region on chromosome 3 (70–160 cM; logarithm of odds [LOD] scores ranging between 1.15 and 2.71), chromosome 4q31 (peak LOD 146 cM; LOD scores ranging between 0.90 and 2.41), and on chromosome 14p (peak LOD 23 cM; LOD scores ranging between 1.43 and 2.31). Ordered subset analysis (OSA) suggests that the linked chromosome 3 region consists of at least two separate loci on 3p and 3q. In addition, OSA based on lipid measures and other traits identify family subsets with significantly stronger evidence of linkage (e.g., CVD2 on chromosome 3 at 87 cM subsetting on low HDL with an initial LOD of 2.19 is maximized to an LOD of 7.04 in a subset of 25% of the families and CVD2 on chromosome 14 at 22 cM subsetting on high triglycerides with an initial LOD of 1.99 maximized to an LOD of 4.90 in 44% of the families). When subjects are defined as affected by the presence of each trait (type 2 diabetes, metabolic syndrome, CVD1, and CCP), significant evidence for linkage to the 3p locus is observed with a peak LOD of 4.13 at 87 cM. While the correlated nature of the traits makes it unclear whether these loci represent distinct type 2 diabetes, metabolic syndrome, or CVD loci or single loci with pleiotropic effects, the coincident linkage suggests that identification of the underlying genes may help clarify the relationship of diabetes, metabolic syndrome, and CVD.
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Diabetes has been widely recognized as an independent risk factor for the development of clinical atherosclerotic cardiovascular disease (CVD) (1–5). For example, the relative risk of cardiovascular death was 2.1 for men and 4.9 for women, comparing diabetes-affected with nondiabetic subjects in the Framingham Study (1). Diabetes substantially contributes to the development of premature mortality and morbidity from CVD and atherosclerotic heart disease, and patients with diabetes are at increased risk of mortality from coronary heart disease (6). In individuals with diabetes, genetic susceptibility as well as other factors (hypertension, microalbuminuria, blood glucose control, etc.) ultimately culminates in a diffuse disease process: diabetic macrovascular disease. These complications are common, afflicting the majority of diabetes-affected individuals. CVD accounts for 65% of deaths in diabetes-affected individuals, and in the state of North Carolina alone diabetes accounts for a quarter of all CVD hospitalizations, costing $629 million in hospital charges in 2000 (7) and representing a 50% increase from 1997. For a problem of such magnitude, little is known about the underlying biological basis for the association of diabetes and CVD. The identification of genetic components and their correlation with environmental risk may help focus on both treatment and intervention strategies.

While the relationship between CVD risk and type 2 diabetes risk has been extensively documented, the relationship of incidence and progression of diabetes and macrovascular disease has not been adequately addressed.

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CarCP, carotid calcified plaque; CCP, coronary calcified plaque; CT, computed tomography; CVD, cardiovascular disease; ECG, electrocardiogram; LOD, logarithm of odds; MI, myocardial infarction; NPL, nonparametric linkage; OSA, ordered subset analysis.

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TABLE 1
Demographic characteristics of Diabetes Heart Study participants (Caucasians)

Phenotype	Diabetes-affected subjects	Nondiabetic subjects
n	810	167
Sex (% female)	51.0	62.3
Age (years)	62.0 ± 9.3	59.6 ± 10.0
Metabolic syndrome affected (%) (*n* = 808)	89.6	49.1
Self-reported prevalent CVD (CVD1) (%) (*n* = 393)	46.4	23.7
Self-reported prevalent CVD (CVD2) and/or CCP >400 (%) (*n* = 589)	67.2	41.8
CCP >0 (%) (*n* = 731)	94.8	84.4
CCP >0 (%) (*n* = 667)	78.2	58.7
Hypertension (hypertensive or taking medication) (%)	71.0	44.2
Duration of diabetes for affected (years)	10.4 ± 7.4	NA
BMI (kg/m^2)	32.4 ± 6.8	28.8 ± 5.1
Waist circumference (cm)	107.6 ± 17.9	95.8 ± 13.6
Total cholesterol (mg/dl)	187.1 ± 43.5	195.0 ± 34.7
Non-HDL cholesterol (mg/dl)	144.9 ± 42.3	146.9 ± 33.1
HDL cholesterol (mg/dl)	42.2 ± 12.0	47.9 ± 13.7
LDL cholesterol (mg/dl)	103.8 ± 31.6	114.1 ± 29.3
Triglycerides (mg/dl)	213.9 ± 142.2	165.0 ± 83.4
A1C (%)	7.7 ± 1.8	5.6 ± 0.51
Fasting glucose (mg/dl)	150.8 ± 58.1	95.0 ± 14.8
C-reactive protein (mg/dl)	1.10 ± 0.30	1.02 ± 0.21

Data are means ± SD or means.

Risks for CVD and type 2 diabetes are widely accepted as being due to genetic and environmental risk factors; indeed, many of the risk factors for CVD and type 2 diabetes are thought to be determined (at least in part) by underlying genetic factors (e.g., insulin resistance and obesity).

Several hypotheses have been put forth to explain the relationship between CVD and diabetes. Some reports indicate that the primary biochemical abnormality of diabetes, i.e., chronic hyperglycemia, directly (and independently of other CVD risk factors) leads to an increased risk of CVD (8). In an opposing hypothesis, the atherogenic risk factor profile in diabetes, and not glucose per se, is implicated. Diabetes is known to worsen CVD risk factors such as insulin resistance, obesity, HDL cholesterol, and blood pressure. All of these factors are known to contribute to an increased risk of CVD. Others (9,10) have challenged these hypotheses with a theory that CVD is not a consequence of diabetes but that these two conditions share common antecedents. The insulin resistance or metabolic syndrome has been hypothesized as one such common antecedent (9). Another theorized common antecedent is genetic susceptibility (9,10). That is, a single gene or several genes are responsible for both chronic diseases.

The Diabetes Heart Study (11,12) is a study of genetic and environmental factors of CVD in families highly enriched for type 2 diabetes. It represents one of the few, if not the only, study in which diabetes and multiple measures of CVD, including vascular calcification, can be simultaneously evaluated to assess the relationship between genetic and environmental contributions. In this report, we describe linkage analysis of discrete traits: type 2 diabetes, metabolic syndrome, and multiple CVD measures.

RESEARCH DESIGN AND METHODS

The Diabetes Heart Study is being conducted in Forsyth County, North Carolina, to study the genetic and epidemiological origins of CVD in families affected with type 2 diabetes. Siblings concordant for diabetes were recruited from internal medicine clinics, endocrinology clinics, and community advertising. Type 2 diabetes was defined as a clinical diagnosis of diabetes after the age of 34 years, in the absence of historical evidence of diabetic ketoacidosis, and active treatment at the time of examination. Unaffected siblings, similar in age to the siblings with type 2 diabetes, were also invited to participate, as were any additional diabetes-affected siblings. The sample includes Caucasian and African-American (~15% of the total) participants. The results reported here are from 977 Caucasian subjects from 358 pedigrees with at least two individuals with type 2 diabetes (575 type 2 diabetic relative pairs, 1,122 total relative pairs). The family structures of the subjects (both affected and unaffected in each family) included in the genome scan are 216 families with 2 sibs, 80 families with 3 subjects, 34 families with 4 subjects, 14 families with 5 subjects, 7 families with 6 subjects, 4 families with 7 subjects, 2 families with 8 subjects, and 1 family with 10 subjects. For the 216 families with two sibs, each sib has type 2 diabetes. The larger families have combinations of type 2 diabetes–affected and –unaffected relatives, but each has at least one pair of type 2 diabetes–affected sibs.

Individuals with serious health conditions, e.g., renal replacement therapy, were not eligible to participate. Recruitment was based upon family structure, and there were no inclusions/exclusions based on prior or current evidence of prevalent CVD at the time of recruitment.

Clinical evaluation. The participant examinations were conducted in the General Clinical Research Center of the Wake Forest University Baptist Medical Center and included interviews for medical history and health behaviors, anthropometric measures, resting blood pressure, a fasting blood draw, and a spot urine collection. Laboratory assays included urine albumin and creatinine, total cholesterol, non-HDL cholesterol, LDL, HDL, triglycerides, HbA_{1c} (A1C), fasting glucose, and blood chemistries. A detailed medical history was collected with emphasis on CVD.

In addition, a resting 12-lead electrocardiogram (ECG) was performed to assess history of clinically significant (past or present) CVD. All ECGs were performed in the General Clinical Research Center and read to detect clinical (or subclinical) evidence of coronary heart disease. ECGs were recorded on a Marquette ECG machine and transmitted to the ECG processing stations of the EPICARE center at Wake Forest University for coding, following standardized extensively tested procedures using the Novacode ECG program to produce quality grades of each ECG received as a part of the overall quality control process. Prevalent ECG abnormalities are classified according to the Minnesota Code (13).

Calcified atherosclerotic plaque, coronary calcified plaque (CCP), and

% trait B given the presence of trait A

% trait A given the presence of trait B		B: T2DM	MS	CVD1	CVD2	CCP	CarCP
A	T2DM		90	44	65	95	79
	MS	90		43	62	95	77
	CVD1	91	88		100*	97	87
	CVD2	89	85	67		98	87
	CCP	84	83	42	64		77
	CarCP	86	84	46	71	96	

*all individuals with CVD1 are included in CVD2

FIG. 1. Concordance of discrete traits in the Diabetes Heart Study subject population.

TABLE 2
Summary of subject pairs available for linkage analysis by trait

Relationship	T2DM	MS	CVD1	CVD2	CCP	CarCP	Super phenotype*
Full sibling	545	646	171	380	826	535	186
Half sibling	13	14	0	4	18	4	4
Avuncular	13	11	0	0	5	0	0
First cousin	4	4	0	0	0	0	0
Parent/offspring	7	5	0	0	1	0	0

*Superphenotype: presence of type 2 diabetes, metabolic syndrome, CVD1, and CCP (all present). MS, metabolic syndrome; T2DM, type 2 diabetes.

carotid calcified plaque (CarCP) were measured in the appropriate arterial bed using single and multidetector computed tomography (CT) systems using a standardized protocol based on those currently implemented in the National Heart, Lung, and Blood Institute's CARDIA (Coronary Artery Risk Development in Young Adults) and MESA (Multiethnic Study of Atherosclerosis) studies (14,15). Technical aspects of the CT exam have been described in detail (16,17). Images were obtained during suspended respiration and with ECG gating at 50% of the RR interval. A calibration standard (Image Analysis, Columbia, KY) was placed underneath each participant during the scans, and in addition to the daily calibrations, biweekly calibration checks for measurement of calcium hydroxyapatite were performed and recorded to document stability of the CT systems' measurement over time. The SmartScore software package (GE Advantage for Windows) was used to analyze the image data by experienced analysts producing measures of calcified plaque mass, volume, and the Agatston score corrected for slice thickness. Calcified plaque was measured using two thresholds for the presence of calcified plaque, a more sensitive 90 CT number threshold, and the conventional 130 CT number threshold.

Metabolic syndrome was defined using criteria established in the third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment in Adults (Adult Treatment Panel III) as the presence of three or more of the following risk factors: waist circumference >88 cm in women and 102 cm in men, triglycerides ≥150 mg/dl, HDL <50 mg/dl in women and 40 mg/dl in men, blood pressure ≥130/85 mmHg, and fasting glucose ≥110 mg/dl or a diagnosis of diabetes (18).

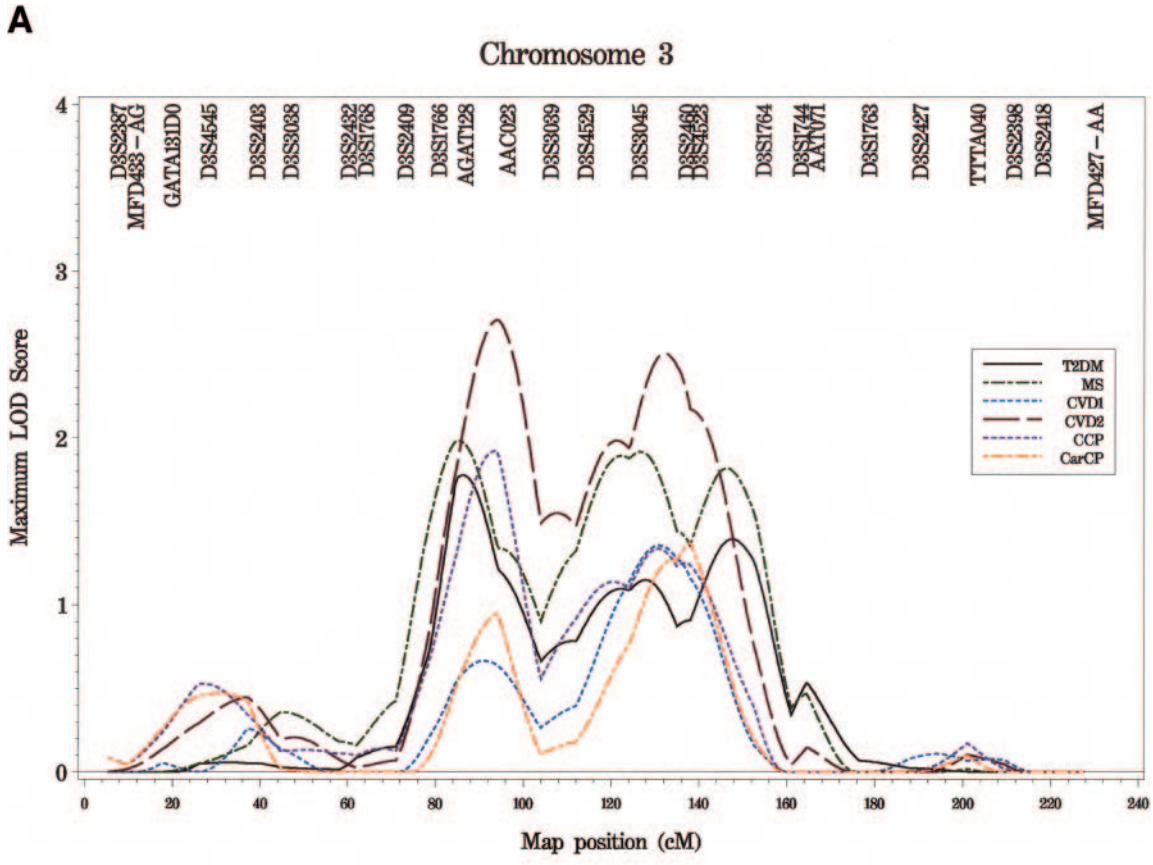
Genotyping. DNA extraction was performed using the PureGene system (Gentra Systems, Minneapolis, MN). A genome-wide scan was completed by the Mammalian Genotyping Service (Marshfield, WI). A total of 1,177 samples from self-reported Caucasian and African-American subjects and 411 polymorphic markers were analyzed, giving a total of ~494,022 genotypes. The results reported here are from 977 Caucasian subjects from 358 pedigrees. All of the markers were taken from screening set 13 (19), which largely consists of tetra- and trinucleotide repeats at an average spacing of 9.3 cM and no intermarker gaps >9.9 cM.

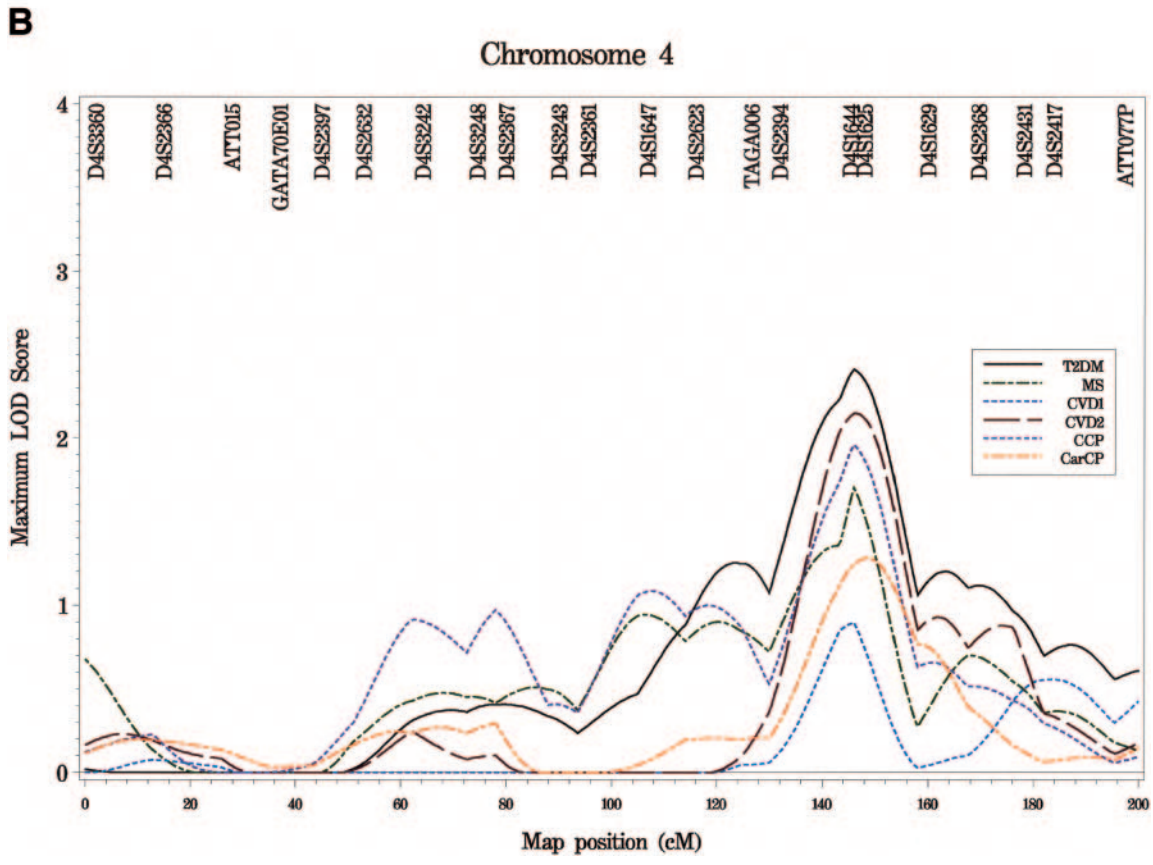
Each pedigree was examined for consistency of familial relationships using PREST (Pedigree Relationship Statistical Test) (20). When the self-reported familial relationships were inconsistent with that determined from the observed genotypic data for that pedigree, then *1*) the pedigree was modified when the identity-by-descent statistics suggested a very clear alternative or *2*) a minimal set of genotypic data were converted to missing. A total of 16 pedigrees exhibited probable incorrect familial relationships and were modified as above, with 15 of these changes being from a full-sibling to half-sibling relationship. Each genetic marker was also examined for Mendelian inconsistencies using PedCheck (21) and sporadic problem genotypes converted to

TABLE 3
NPL regression analysis of genome scan data: LOD scores >1.25

Chromosome	Trait	Position	LOD	LOD-1 interval	*P* value*	Nearest marker
3	MS	85	1.98	75–103	0.003	AGAT128
3	T2DM	86	1.78	79–102.5	0.004	AGAT128
3	CCP	93.5	1.92	81–101.5	0.003	AAC023
3	CVD2	94	2.71	83.5–103	0.0004	AAC023
3	MS	126.5	1.92	104–157.5	0.003	D3S3045
3	T2DM	128	1.15	71–174	0.021	D3S3045
3	CCP	130.5	1.34	74–153.5	0.013	D3S3045
3	CVD1	131	1.36	109–149.5	0.012	D3S3045
3	CVD2	132	2.51	112–147	0.001	D3S2460
3	CarCP	138	1.37	116–150	0.012	D3S4523
3	MS	146.5	1.82	73.5–158	0.004	D3S4523
3	T2DM	147.5	1.39	74.5–160.5	0.011	D3S4523
4	CCP	107.5	1.09	44.5–193.5	0.025	D4S1647
4	CCP	146	1.96	134.5–155.5	0.003	D4S1625
4	CVD2	146	2.15	136–156.5	0.002	D4S1625
4	MS	146	1.70	99–154.5	0.005	D4S1625
4	T2DM	146	2.41	133–156	0.001	D4S1625
4	CarCP	148.5	1.28	131.5–172	0.015	D4S1625
6	CCP	103	1.43	85.5–115	0.010	D6S1056
6	T2DM	106	1.27	71–122	0.016	D6S1056
6	MS	112	1.06	68–124	0.027	D6S1021
7	CCP	41.9	1.29	23.9–57.9	0.015	D7S1808
10	CVD2	136	1.30	120.5–153.5	0.014	D10S1425
13	MS	64	1.82	35.5–74	0.004	D13S317
14	T2DM	22	1.48	0–33.5	0.009	ATA77F05
14	CarCP	23	1.43	0–46	0.010	ATA77F05
14	CVD2	23	2.13	14.5–31	0.002	ATA77F05
14	MS	23	2.31	13–27.5	0.001	ATA77F05
14	CVD1	27	1.44	0–50.5	0.010	D14S608
20	CVD2	29.6	1.57	0–48.6	0.007	ATTC013

*Based on large-sample approximation. MS, metabolic syndrome; T2DM, type 2 diabetes.





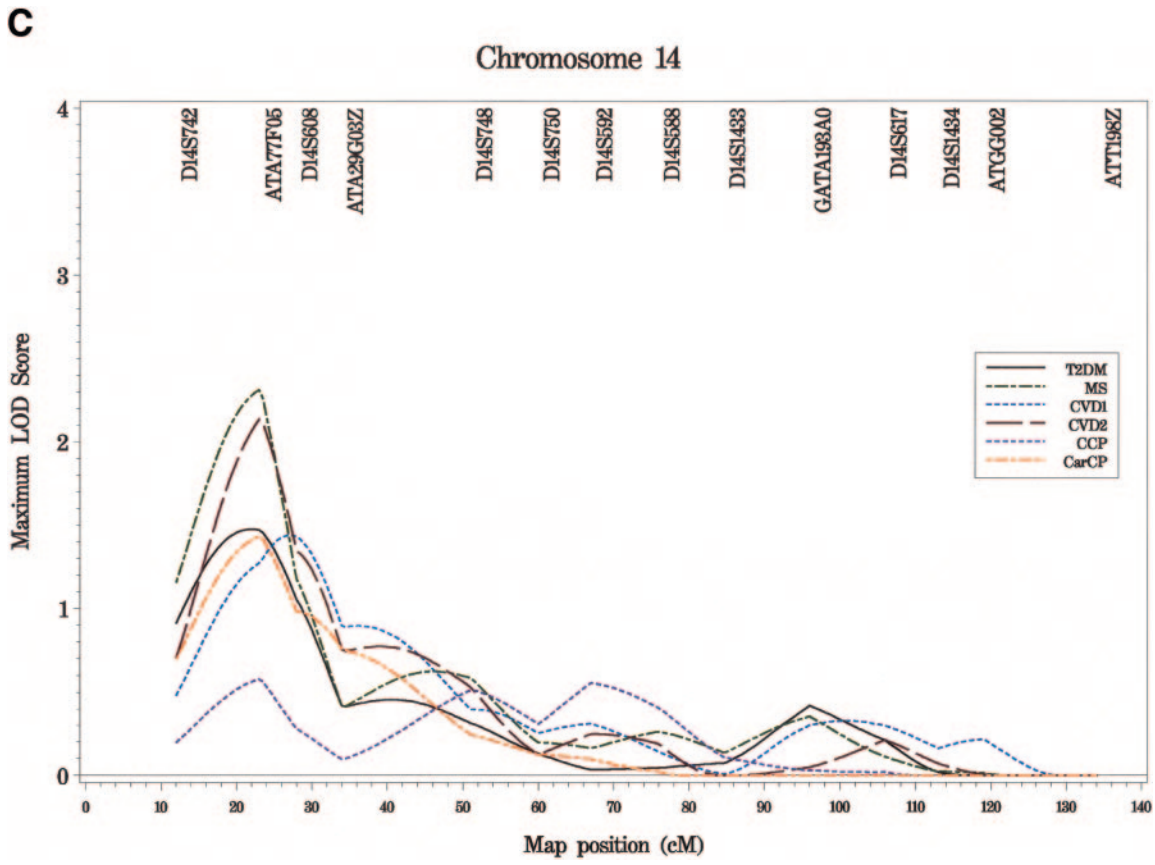


FIG. 2. NPL graphs of chromosomes showing coincident linkage of discrete traits. Marker locations are shown across the top and map position in cM is shown along the bottom. Curves for individual traits are color coded as shown within the figures. *A*: Chromosome 3. *B*: Chromosome 4. *C*: Chromosome 14.

missing. A total of 1,079 genotypes were changed, with 1,046 changed to missing and 33 genotypes changed based on electropherograms provided by the Mammalian Genotyping Service. Allele frequency estimates were derived from the genome scan genotyping data from the families through computing the maximum likelihood methods implemented in the software Recode (D. Weeks, personal communication). Map distances were based on the Marshfield genetic map (22).

Linkage analyses. Multipoint linkage analyses were carried out using nonparametric linkage (NPL) regression analyses using the NPL_{pairs} statistics outputted from a modified version of Genehunter (23–26). Ordered subset analyses (OSAs) (27) were computed to investigate the influence of quantitative traits on evidence of linkage of the discrete traits under study. In this analysis, the following clinical measures were assessed: lipids (total, HDL, LDL, and non-HDL cholesterol and triglycerides), measures of glycemic control (fasting glucose and A1C), inflammation (C-reactive protein), and body size (BMI and waist circumference). OSA ranks each family by the family-level value of a covariate of interest and identifies the contiguous subset of families that maximize the evidence for linkage. Details of the application of this method in our laboratory have been previously described (28). For the purposes of this study, the family mean for ordering subsets was calculated from affected subjects (i.e., type 2 diabetes or metabolic syndrome, etc.) only. The statistical significance of the change in the LOD score was evaluated by a permutation test under the null hypothesis that the ranking of the covariate is independent of the family's LOD score on the target chromosome. Thus, the families were randomly permuted with respect to the covariate ranking, and an analysis was performed for each permutation of these data. The resulting empirical distribution of the change in the LOD scores yielded a chromosome-wide *P* value. The family-level means were ranked in ascending order and then the analysis repeated in descending order.

RESULTS

Marker data from the genome scan was evaluated for evidence of linkage to the discrete traits of type 2 diabetes, metabolic syndrome, presence/absence of CCP, presence/absence CarCP, and measures of prevalent CVD. Diagnosis of type 2 diabetes and metabolic syndrome are described in RESEARCH DESIGN AND METHODS. A descriptive summary of these and related traits for the study subjects is shown in Table 1. Consistent with a diabetes-enriched sample, the subjects in the study have high rates of metabolic syndrome, hypertension, dyslipidemia, obesity, and evidence of subclinical or clinical CVD. It should be noted also that the nondiabetic siblings of type 2 diabetes–affected subjects have high rates of these clinical disorders also, e.g., 49% have metabolic syndrome and 46% have hypertension.

Presence of CVD in subjects was estimated in two ways: presence of subclinical disease and evidence of prevalent clinical disease. A major focus of the Diabetes Heart Study is evaluation of subclinical CVD using calcified atherosclerotic plaque measured using CT. CCP is a predictor of prevalent CVD and total mortality (29–33). In addition, the study has collected data from other vascular beds such as CarCP. For the purposes of this report, subjects are dichotomized based on presence or absence of detectable calcified plaque in the appropriate vascular bed. Two measures of prevalent CVD, CVD1 and CVD2, were included in the analysis. CVD1 was based on subject self-reported CVD. Criteria for CVD1 were one or more of the following: history of hard events, i.e., myocardial infarction (MI) or stroke, procedures (coronary artery bypass graft, endarterectomy, or angioplasty), silent MI based on ECG, or angina. A total of 393 subjects had CVD1. The largest category for assignment to CVD1 was subjects with a previous history of both events and procedures ($n = 228$, 56% of CVD1), and the least definitive basis for assignment was the presence of angina alone ($n = 43$, 11% of CVD1).

TABLE 4
OSA on chromosomes 3,4, and 14

Phenotype	Linked subset*	Position (cM)	Nearest marker	Initial LOD	Max LOD	Subsetting variable trait: Optimal subset	Subsetting variable trait: Remaining families	Empirical *P* value	Percentage of linked families
Chromosome 3p									
CCP	HDL cholesterol (S)	85	AGAT128	1.37	5.19	36.5 ± 7.0	46.3 ± 13.3	0.046	31
	Non-HDL cholesterol (S)	85	AGAT128	1.32	5.39	127.8 ± 24.8	151 ± 43.2	0.040	28
CVD2	HDL cholesterol (S)	87	AGAT128	2.19	7.04	37.5 ± 7.1	45.2 ± 13.4	0.006	25*
Chromosome 3q									
CarCP	C-reactive protein (H)	130	D3S2460	1.31	3.16	1.28 ± 1.47	0.32 ± 0.48	0.058	35
CCP	HDL cholesterol (L)	132	D3S2460	1.30	3.87	34.0 ± 7.1	47.4 ± 12.3	0.044	34
CVD1	LDL cholesterol (S)	124	D3S4523	1.01	4.04	99.5 ± 21.9	109 ± 35.1	0.041	30*
	A1C (H)	129	D3S4523	1.36	3.41	8.03 ± 1.94	6.44 ± 1.11	0.015	66*
	A1C (S)	134	D3S1764	1.36	4.26	7.73 ± 1.60	6.93 ± 1.91	0.026	58*
CVD2	A1C (H)	133	D3S1764	2.60	4.91	7.76 ± 1.87	6.27 ± 1.04	0.017	80*
	A1C (S)	134	D3S1764	2.57	5.57	7.69 ± 1.59	6.94 ± 1.94	0.050	60*
Metabolic syndrome	HDL cholesterol (L)	148	D3S1764	1.82	5.03	34.5 ± 7.28	47.6 ± 12.4	0.0036	36*
	HDL cholesterol (S)	148	D3S1764	1.83	5.20	34.8 ± 7.1	47.4 ± 12.6	0.025	35*
Chromosome 4									
CarCP	Non-HDL cholesterol (H)	144	D4S1644	1.17	3.21	171 ± 41.6	128 ± 29.6	0.026	41
CCP	Waist circumference (H)	146	D4S1625	2.05	3.86	119 ± 19.2	99.2 ± 12.6	0.041	37
	A1C (H)	147	D4S1625	2.02	3.43	7.9 ± 1.9	6.3 ± 1.0	0.05	72
CVD1	Triglycerides (H)	146	D4S1625	1.10	2.77	272 ± 161	145 ± 62	0.0353	49*
CVD2	HDL cholesterol (L)	144	D4S1625	2.31	4.02	37.8 ± 8.5	51.0 ± 13.4	0.0214	62*
Metabolic syndrome	A1C (H)	146	D4S1625	1.76	4.35	7.89 ± 1.90	6.36 ± 1.08	0.004	73*
	A1C (S)	146	D4S1625	1.76	4.35	7.89 ± 1.90	6.36 ± 1.08	0.0277	73*
	Non-HDL cholesterol (H)	146	D4S1625	1.89	3.63	176 ± 44	132 ± 31	0.032	31*
Type 2 diabetes	Waist circumference (H)	146	D4S1625	2.47	4.31	117 ± 18.9	98.6 ± 12.5	0.03	45
Chromosome 14									
CarCP	Triglycerides (H)	25	ATA77F05	1.39	4.74	288 ± 170	151 ± 63.9	0.0006	42
	Triglycerides (S)	32	ATA29G03Z	0.93	4.97	273 ± 141	165 ± 113	0.013	39
CCP	Triglycerides (H)	21	D14S742	0.50	2.77	283 ± 167	148 ± 61.0	0.036	44
CVD1	A1C (H)	17	D14S742	0.94	3.77	8.55 ± 2.13	6.66 ± 1.14	0.010	43*
CVD2	Triglycerides (H)	22	ATA77F05	1.99	4.90	283 ± 167	150 ± 64.4	0.0039	44*
	Triglycerides (S)	22	ATA77F05	1.99	4.90	282 ± 167	150 ± 64	0.030	44*
	Waist circumference (cm) (S)	23	ATA77F05	2.24	6.06	104 ± 11.4	106 ± 19.4	0.008	27*
	Non-HDL cholesterol (H)	23	ATA77F05	2.10	3.53	173 ± 43.1	130 ± 30.1	0.044	37*
Metabolic syndrome	Triglycerides (H)	24	ATA77F05	2.25	4.22	286 ± 169	151 ± 65	0.014	42*
	C-reactive protein (L)	23	ATA77F05	2.33	4.16	0.37 ± 0.37	1.53 ± 1.77	0.019	78*
	C-reactive protein (S)	26	D14S608	1.82	4.75	0.54 ± 0.45	0.68 ± 1.24	0.049	35*
Type 2 diabetes	Triglycerides (H)	38	ATA29G03Z	0.72	3.46	285 ± 169	151 ± 65.5	0.022	43

Traits with $P < 0.05$ and included ≥25% families. *Subset code: H, high; L, low; S, optimal slice.

Of the subjects with angina alone, 15 had CCP scores >400. The trait CVD2 was defined as participants with CVD1 or with CCP score >400. CCP score of >400 was chosen to be consistent with previous reports that have used this level of CCP as a definition of high risk for CVD (34,35). Addition of CCP >400 resulted in an additional 201 subjects for a total of 606 with CVD2.

The primary phenotypes of type 2 diabetes, metabolic syndrome, CCP, CarCP, CVD1, and CVD2, are frequently present together in subjects. This is illustrated in Fig. 1, which shows percent coincidence of these traits in subjects. Depending upon the direction and trait, coincidence ranges from a low of 42% of subjects in the CVD1 category that have detectable CCP to >90% for multiple combinations, e.g., 95% of subjects with type 2 diabetes have detectable CCP.

A multipoint linkage analysis was carried out and multipoint LOD score curves for each chromosome were generated. A summary of subject pairs available for linkage analysis by trait is shown in Table 2. The genomic locations for each trait in which the maximum LOD score exceeded 1.25 are presented in Table 3, which lists trait, LOD score, and genomic location. With the exception of a small number of nominally positive LOD scores (CCP on chromosome 7, CVD2 on chromosome 10, metabolic syndrome on chromosome 13, and CVD2 on chromosome 20) the majority of noteworthy LOD scores cluster in the same locations on chromosomes 3, 4, and 14 and, to a lesser degree, on chromosome 6. Type 2 diabetes, metabolic syndrome, CVD1, CVD2, CCP, and CarCP map to a broad region on chromosome 3 (70–160 cM; LOD scores between 1.15 and 2.71), chromosome 4q31 (peak LOD 146 cM; LOD scores between 0.90 and 2.41), and chromosome 14p (peak LOD 23 cM; LOD scores between 1.43 and 2.31). Figure 2 shows LOD score curves for chromosomes 3, 4, and 14. The pattern of linkage results with multiple traits is highly suggestive of coincident linkage of the discrete traits to the same chromosomal regions. This is especially true for chromosomes 4 and 14 (Fig. 2*B* and *C*). Chromosome 3 (Fig. 2*A*) presents a more complex pattern with nominal evidence of linkage over an extensive distance (70–160 cM), which is broadly consistent with the possibility of two or more peaks.

The data were subjected to further analysis using OSA,

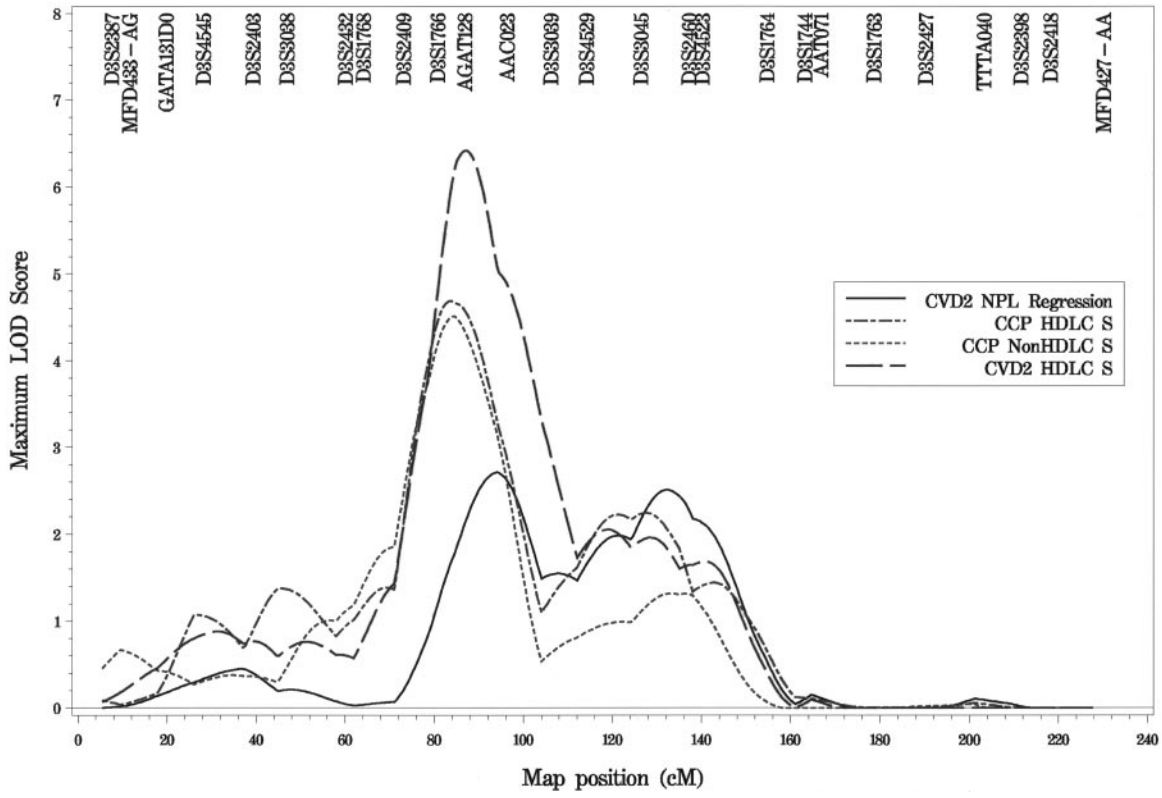


FIG. 3. OSA of chromosome 3 evaluating linkage to CCP and CVD2 subsetting families based on mean HDL cholesterol and non-HDL cholesterol. Continuous black line is initial NPL regression analysis. Broken colored lines are OSA analyses.

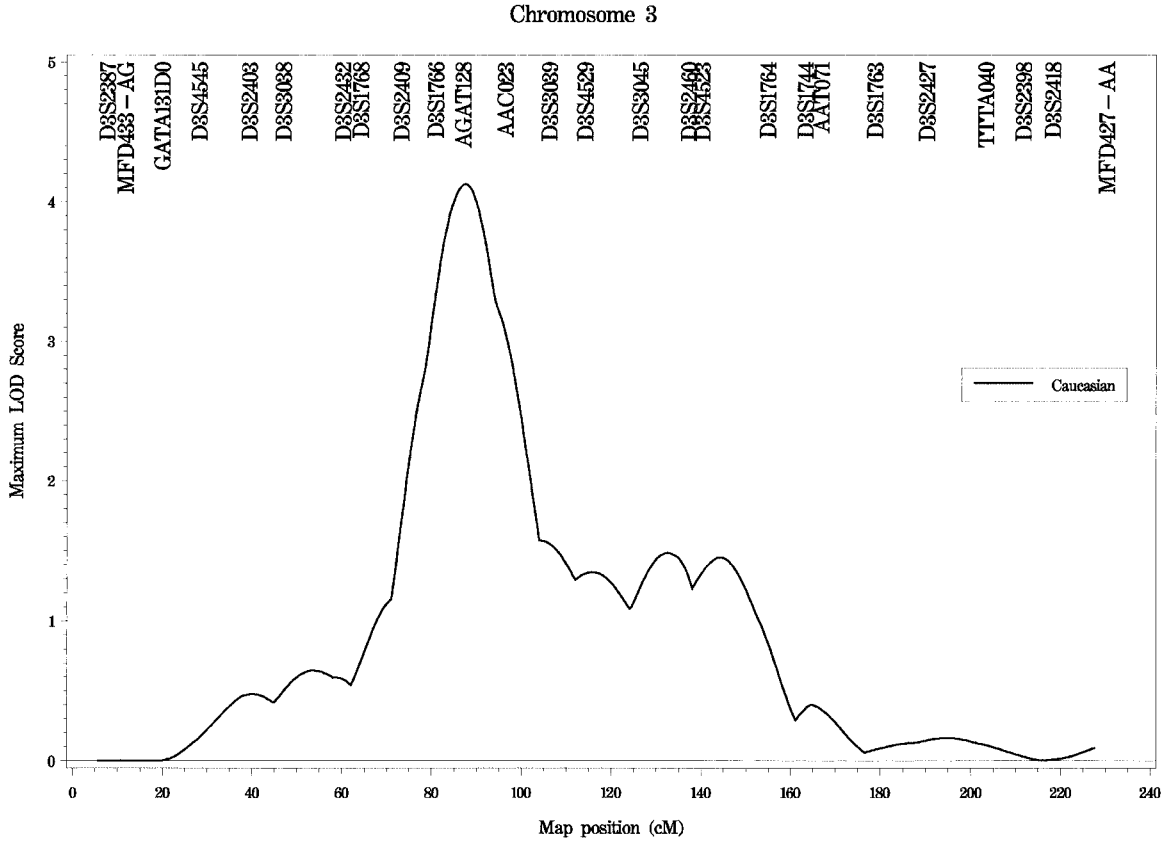


FIG. 4. NPL linkage analysis results for chromosome 3 in which affected status is defined as presence of type 2 diabetes, metabolic syndrome, CVD1, and CCP.

an approach that can be used to evaluate linkage under assumptions that it will be more readily detected in subgroups of families in a population that are differentiated by specific phenotypic traits. This approach was used to search for differential evidence for linkage depending on quantitative traits available in each subject. Table 4 summarizes the results of OSA in this dataset, in which the estimated maximum LOD score was significantly higher ($P \leq 0.05$) than the initial LOD score, and the percentage of linked families in the subset contributing to the maximum LOD score was >25% of the total families. OSA results summaries are grouped by linkage locus. The number of traits and locations is large, but several observations can be made. First, ordered subsets can be identified that result in substantial LOD scores. For example, in mapping CVD2 on chromosome 3p and subsetting on lower levels of HDL, a subset consisting of 25% of the families results in a maximized LOD score of 7.04 at 87 cM in comparison to an LOD of 2.19 in the total sample. Mapping CarCP on chromosome 14, subsetting on higher levels of triglyceride, a subset consisting of 42% of the families results in a maximized LOD score of 4.74 at 25 cM and, in the same region of chromosome 14 at 22 cM and again subsetting on high triglycerides, a maximization to an LOD of 4.90 in 44% of the families was observed compared with an initial LOD of 1.99 for CVD2.

There are relatively few OSA results for the chromosome 3p locus that are significant (Table 4), but the pattern of a proximal 3p locus and a distal 3q locus are broadly consistent with results from the unstratified analysis. Significant subsets are seen on 3p, ~85–87 cM for CCP and CVD2 subsetting on lower HDL and non-HDL cholesterol, with maximum OSA LOD scores of 5.19–7.04 in 25–31% of the families. Figure 3 shows the OSA analysis of CCP and CVD2 on chromosome 3 when subsetting on low or optimal-slice HDL or non-HDL cholesterol compared with the result observed with the initial NPL regression analysis. A similar pattern is observed for several traits in the 3q region with maximum LODs 124–148 cM subsetting on high C-reactive protein, low HDL, high LDL, and high A1C. High C-reactive protein, low HDL, high LDL, and high A1C are consistent with family subsets that have a greater CVD risk factor profile. It should be noted that these patterns appear even with the potentially confounding influences of extensive use of hypertension and lipid-lowering medications in the subjects (e.g., 44% of diabetes-affected and 28% of unaffected subjects on lipid-lowering medication).

OSA analysis results for chromosome 4 are also summarized in Table 4 and present a consistent picture of subsets with significantly increased LOD scores maximizing at 144–147 cM, corresponding to the 4q31 linkage peak observed in unconditional analysis (Fig. 2*B*). Similar to chromosome 3 results, increased non-HDL cholesterol, waist circumference, A1C, and lower HDL lead to subsets with significantly higher LOD scores for CarCP, CCP, CVD1, CVD2, metabolic syndrome, and type 2 diabetes. Significant OSA LOD scores range up to 4.31 for type 2 diabetes linkage in 45% of the families with increased mean waist circumference.

OSA analysis of chromosome 14 resulted in a consistent pattern of high-triglyceride subsets for all six mapped traits: type 2 diabetes, metabolic syndrome, CVD1, CVD2, CCP, and CarCP, with a small number of other significant subsets. For the high-triglyceride subsets, the maximum LOD scores were observed between 17 and 38 cM. OSA LOD scores for the high-triglyceride subsets range from 2.77 to 4.90, and optimal-slice analysis leads to a high OSA LOD of 4.97 for CarCP. It is also noteworthy that in CVD2 analysis, subsetting on multiple traits leads to significantly increased LOD scores maximizing 21–23 cM, with a high OSA LOD score for CVD2 observed with an optimal-slice subset of high–waist circumference families (27% of families, OSA LOD of 6.06).

Due to the coincidence of the discrete traits (Fig. 1), we performed additional linkage analyses by creating a single "super" phenotype defining affected status by the presence of type 2 diabetes, metabolic syndrome, CVD1, and CCP. Thus, in order to be considered affected in this analysis, a subject had to have a diagnosis of type 2 diabetes and metabolic syndrome with a history of prevalent CVD and detectable CCP. Based on this definition of affected status, 443 of 977 subjects with complete data for all four traits were affected. In this linkage analysis with the combined trait, evidence for linkage in the 3q, 4q, and 14p regions did not substantially change; however, as shown in Fig. 4, a striking increase in the LOD score for the 3p locus was observed with a maximum LOD of 4.13 at 87 cM. This is compared with a maximum LOD of 2.71 for CVD2, the highest LOD score observed in the single-trait analysis. It should be emphasized that this is a result from the NPL regression analysis (i.e., is not the result of OSA). OSA analysis using the variables described above and this super phenotype did not reveal any subsets that resulted in significantly increased evidence for linkage with this 3p peak. This is perhaps not surprising since the magnitude of the LOD score effectively limits the potential to find ordered subsets with significantly higher LOD scores. OSA analyses of the super phenotype at the 3q, 4q, and 14p loci do lead to significant increases in LOD score comparable with those seen for individual traits (data not shown).

DISCUSSION

We have carried out a genome scan of discrete traits in the Diabetes Heart Study, a study of diabetes-enriched families with multiple measures of CVD. The qualitative traits of type 2 diabetes, metabolic syndrome, prevalent CVD (as measured by two definitions), presence of CCP, and presence of CarCP show a consistent pattern of coincident linkage in four chromosomal regions: 3p, 3q, 4q, and 14p. Ordered subset linkage analysis of the data reveals subsets of the families that have LOD scores that range up to an LOD of 5–6 in some cases. When affected status is defined as the combined presence of type 2 diabetes, metabolic syndrome, prevalent CVD (CVD1), and presence of CCP, a significant LOD score of 4.13 was observed in the chromosome 3p region. The evidence of coincident linkage for these traits is striking and has not previously been observed. Few other family-based studies have such comprehensively phenotyped subjects; neither, to our knowledge, do other studies have such an extensive collection of type 2 diabetic families. While the correlated nature of the traits makes it unclear whether these loci represent distinct type 2 diabetes, metabolic syndrome, or CVD loci or single loci with pleiotropic effects, the coincident linkage suggests that identification of the underlying genes may help clarify the relationship of CVD, diabetes, and metabolic syndrome.

Interpretation of these results is a challenge due to the high level of concordance of the traits that have been examined (Fig. 1). At least two simple models seem

possible. In one model, individual genes, for example a gene on 4q, may have a pleiotropic effect that contributes directly to the development of each phenotype type 2 diabetes, metabolic syndrome, CVD1, CVD2, CCP, and CarCP. An alternative model is that these loci represent linkages to a discrete trait, e.g., type 2 diabetes or CVD, which, in the context of the type 2 diabetic family–based study design, will also inherently be reflected in evidence for linkage to the other traits. Broadly consistent with the first model is the observation that when using the stringent criteria for affected status of type 2 diabetes plus metabolic syndrome plus CVD1 plus CCP, compelling evidence for linkage (maximum LOD of 4.13) is observed at the 3p locus (Fig. 4). Thus, a homogeneous subset of "multiply affected" subjects leads to increased evidence of linkage at least at one locus. Consistent with the second model is the observation that in OSA, ordering on well-recognized CVD risk factors (e.g., cholesterol, LDL, HDL, triglycerides) leads, in many cases, to identification of subsets of families that exhibit substantial increases in evidence for linkage. A striking example of this is that ordering on high-triglyceride subsets of families leads to substantially increased evidence of linkage at the 14p locus. These are simple models so alternatives are possible, and, with at least four loci involved, different models may be more or less relevant for each locus. A clearer understanding of these loci will likely be possible when the trait-defining genes are ultimately identified. Despite our inability to clearly define the nature of these mapped loci, the potential significance to understanding the relationship of type 2 diabetes, metabolic syndrome, and CVD that would result from identification of the genes underlying them is clear.

CCP and CarCP are traits that can also be evaluated as continuous, i.e., quantitative traits. The question immediately arises whether the results of linkage analysis of CCP and CarCP as continuous traits lend support to the linkage results reported here. Analysis of the quantitative traits is ongoing, but broadly speaking there is support between the discrete and quantitative trait analysis, e.g., for CCP, LOD is 1.21 at 111 cM on chromosome 3 and for CarCP, LOD is 1.04 at 31 cM on chromosome 14. The strongest evidence for linkage with quantitative analysis of CCP and CarCP is, however, at loci different from the 3p, 3q, 4q, and 14p loci described here. This may not be surprising in light of previous analyses (11) in the Diabetes Heart Study sample that suggest initiation of vascular calcification, thus presence or absence of CCP and CarCP, and progression of vascular calcification, e.g., quantity of vascular calcification reflect, at least in part, biologically separate genetic susceptibilities.

While the type 2 diabetes, metabolic syndrome, CCP, and CarCP traits are relatively easy to accurately assess in the context of this study, we are aware that definition of prevalent CVD in this study may not be robust, as we relied upon self-report of events and procedures. While there is evidence, especially for procedures, that such self-reports are accurate (36,37), we do not have extensive validation of self-reports in this sample. A majority of the 393 subjects assigned the CVD1 phenotype had both events and procedures, and only 43 subjects (11% of CVD1) were assigned based on reported angina alone. It should be noted that the results of the analysis using CVD1 are quite consistent with other traits mapped in this study. Given the hypothesized relationship between glycemic control in type 2 diabetic patients and CVD, it is particularly interesting to observe that the evidence for linkage to both CVD1 and CVD2 on 3q (Table 4) significantly increases in the pedigrees with the highest A1C. This was among the strongest results in these data, with 66 and 80% of pedigrees contributing to the evidence for linkage for CVD1 and CVD2, respectively.

In a similar vein, while there is an intense current interest in vascular calcification as a marker of subclinical CVD, a consensus on the predictive value of vascular calcification measures has not been reached. It should be noted, however, that numerous reports (33,34,38–40) suggest that vascular calcification, with emphasis on CCP, is a valuable marker of subclinical CVD.

Reference to other genome scans may provide some insight into the identity of the 3p, 3q, 4q, and 14p loci that we have mapped in this study. A very large number of genome scans of type 2 diabetes have now been performed in many different populations. In other type 2 diabetes scans, there is limited evidence of type 2 diabetes mapping to any of these regions with a single report of mapping type 2 diabetes in Hong Kong Chinese near the 4q locus observed in this study (41) (LOD of 2.63 at 135 cM). The number of genome scans for metabolic syndrome are limited and none show evidence of linkage in regions where we have observed evidence of linkage. Genome scans for CVD cover a diverse spectrum of traits including MI and prevalent coronary heart disease, with relatively few scans of carotid plaque and vascular calcification (42–44). Hauser et al. (45) observed evidence of linkage of early-onset coronary artery disease to 3q13 (LOD of 3.3) in the GENECARD study. Wang et al. (46) have performed a genome scan for MI in a primarily Caucasian population and reported evidence of linkage to 4q32 with an LOD of 5.08, a location consistent with the 4q locus linked in this study. In addition, Carr et al. (44) report evidence of linkage from a genome scan of CCP to the 4q region also (LOD of 2.43 at D4S2286).

In summary, the Diabetes Heart Study represents a unique resource for genetic analysis of CVD in diabetes-enriched families. Evidence for coincident linkage of type 2 diabetes, metabolic syndrome, and measures of CVD into four chromosomal loci has been observed, and when traits are mapped as a combined super phenotype, significant evidence for linkage is (LOD = 4.13) is observed on chromosome 3p. It is unclear whether these loci represent linkage to specific traits or single loci with pleiotropic effects, but the coincident linkage suggests that identification of the underlying genes will provide insights into the relationship between diabetes and CVD.

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