A Gene Conferring Susceptibility to Type 2 Diabetes in Conjunction With Obesity Is Located on Chromosome 18p11

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Genome-wide nonparametric linkage analysis of 480 sib-pairs affected with type 2 diabetes revealed linkage to a previously unreported susceptibility locus on chromosome 18p11. This result improved with stringent subphenotyping using age- and sex-adjusted BMI, ultimately reaching a logarithm of odds of 3.82 (allele sharing 0.6654) at a point between markers D18S976 and D18S391 when the most obese 20% of the sample was analyzed. Several genes on chromosome 18 have been suggested as metabolic disease candidates, but none of these colocalize with our linkage result. We conclude that our results provide support for the presence of a currently uncharacterized gene on chromosome 18p, certain alleles of which confer increased susceptibility to type 2 diabetes in conjunction with obesity. We additionally observed moderate evidence for linkage to chromosome 1, near marker D1S3462; chromosome 4, near marker D4S2361; chromosome 5, near marker D5S1505; and chromosome 17, near marker D17S1301. *Diabetes* 50:675–680, 2001

Type 2 diabetes is an adult-onset disorder characterized by breakdown of glucose homeostasis, resulting in glucose intolerance followed by overt fasting hyperglycemia. A suite of physiological manifestations accompany hyperglycemia, including peripheral insulin resistance, hepatic overproduction of glucose, and in many cases obesity.

It has long been understood that genetics play a role in predisposition to type 2 diabetes (1). Mutations giving rise to several rare monogenic forms of this disorder have been cloned, including mutations in the insulin gene and in a number of genes conferring lean early-onset type 2 diabetes (maturity-onset diabetes of the young [MODY]); however, no gene predisposing to the common obese adult-onset phenotype has been identified. One important reason for this is the substantial locus heterogeneity associated with diabetes risk. Despite identification of at least five MODY loci to date, there remain pedigrees that segregate autosomal dominant type 2 diabetes not attributable to detectable mutations in any of these genes (2). Similarly, it has been recognized that as many as 10% of patients diagnosed with type 2 diabetes may instead suffer from a disease etiologically (and presumably genetically) more akin to type 1 diabetes (3).

This report describes a genome-wide linkage analysis designed to identify type 2 diabetes susceptibility loci in pedigrees ascertained from Finland and Sweden. It does not attempt to directly replicate the earlier work of Mahtani et al. (4), who studied only families ascertained within the linguistically distinctive Botnia region of northwestern Finland. Rather, we chose to sample from a broader geographic region within Scandinavia because such a strategy was necessary for collecting a sample large enough to obtain robust evidence for linkage in this complex polygenic trait.

When multiple susceptibility loci exist, different pedigree members may exhibit similar phenotypes having different genetic underpinnings, making inference from allele sharing among affected members problematic. The likelihood of confounding heterogeneity increases with reduction in relatedness among affected pedigree members. To minimize heterogeneity, we ascertained small pedigrees, in which the most distantly related affected members are at most first cousins, and avoided ascertainment of pedigrees in which there was evidence for bilineality. Disease susceptibility alleles may also exhibit incomplete penetrance, allowing identity-by-descent allele sharing between affected and unaffected pedigree members even at "true" susceptibility loci. To avoid the requirement of specifying an (unknown) penetrance function, we have used nonparametric allele-sharing methods (5) exclusively.

Another strategy to reduce genetic heterogeneity is sample stratification, or subphenotypic classification, which is the identification of a phenotypically distinctive subset of affected members whose similarity is explained

TABLE 1
Distribution of variables used for subphenotypic classification in linkage analysis

Phenotype	Global		Affected		Percentile threshold values			
	Means ± SD	Range	Means ± SD	Range	20%	30%	40%	50%
BMI	27.6 ± 4.9	13.9–62.7	29.0 ± 5.0	14.1–62.7	31.7	30.5	29.4	28.6
Waist-to-hip ratio	0.907 ± 0.095	0.563–1.611	0.938 ± 0.086	0.604–1.281	0.986	0.962	0.950	0.933
Age at onset	—	—	52.1 ± 11.5	35–88	47.5	50.3	52.0	54.0

Threshold values reflect pedigree means for affected subjects only.

by greater genetic homogeneity within the subset than exists in the overall sample. In the case of type 2 diabetes, one would ideally stratify on the basis of insulin resistance and/or severity of insulin secretion defect. However, confounding environmental effects, including varying duration of disease, differing access to health care, heterogeneity in prescription, and variation in adherence to treatment regimes, make inferences about insulin action in diabetic patients problematic, especially inferences based solely on oral glucose tolerance test (OGTT) data (6). Therefore, we chose to stratify using age at onset of diabetes—inferring that an earlier onset of disease may indicate greater genetic liability (7)—and two measures of central obesity: waist-to-hip ratio and BMI. Using the latter two measures presumes that only a subset of type 2 diabetes susceptibility loci may predispose to the combination of diabetes and obesity, an assertion supported by epidemiological data suggesting the existence of genetic factors that simultaneously influence abdominal visceral fat and plasma insulin levels (8).

RESEARCH DESIGN AND METHODS

Ascertainment and phenotyping. We recruited participants from Finland and southern Sweden; no subjects participating in the Botnia type 2 diabetes genome scan (4) were included in this study. Diabetes clinics in Finland and southern Sweden were appointed to participate in the study; after a clinic was appointed, information on all type 2 diabetes patients and their family members was obtained by the clinic's nurse and verified from clinic records. This strategy differs from that of the ongoing fusion (Finland–United States Investigation of NIDDM Genetics) type 2 diabetes linkage study in that probands for that study have been identified using hospital discharge records and have been recruited only from Finland, rather than both Finland and Sweden. In our study, 617 families characterized by at least one diabetic individual in addition to the proband were mailed a questionnaire and invitation to participate in the study. Approval was secured from the appropriate institutional review boards (IRBs) for all procedures involving human subjects in both Finland and Sweden. In Finland, approval was granted on 13 June 1994 by the IRB of the Fourth Department of Medicine, Helsinki University Hospital (Dr. Tom Törnroth, chairman) and on 17 June 1994 by the Finland Ministry of Health and Social Welfare (Dr. Kimmo Leppo, chairman). In Sweden, approval was granted on 9 July 1994 and 1 March 1995 by the IRB of Lund University Hospital (Dr. Andres Gustafsson, chairman). Ascertainment was carried out in the following cities in Finland: Helsinki, Lappeenranta, Joensuu, Mikkeli, Rovaniemi, Loviisa, Hämeenlinna, and Pori. In Sweden, it was carried out in Malmö, Karlskrona, Växjö, and Skara.

Altogether, 3,064 people from 555 families (1,664 people from 290 families in Finland and 1,400 people from 275 families in Sweden) agreed to participate and were evaluated; of these, 1,599 people had diabetes according to 1980 World Health Organization (WHO) criteria (fasting plasma glucose ≥7.8 mmol/l or random plasma glucose ≥11.1 mmol/l). The average number of affected members per family was 2.8. To improve phase inference, we ascertained all available unaffected sibs and offspring. Patients underwent extensive phenotyping, including anthropometric measurements and measurement of fasting blood glucose, insulin, and lipids; patients who had not received a physician's diagnosis of diabetes at the time of enrollment were asked to undergo an OGTT.

Samples for measurement of blood glucose and serum insulin were drawn at 0, 30, 60, and 120 min after ingestion of 75 g glucose. Blood glucose was measured by a glucose oxidase method (Hemocue, Sweden), and serum insulin was measured by radioimmunoassay (Pharmacia, Sweden) with an interassay coefficient of variation (CV) of 7.5%. Serum C-peptide was measured by double-antibody radioimmunoassay (Linco) with a CV of 9.0%, and GAD65 antibodies were measured by a modified radiobinding assay using ^{35}S-labeled recombinant human GAD65. Body weight and height were measured with subjects in light clothing without shoes. With the subjects standing, waist circumference was measured with a soft tape midway between the lowest rib and the iliac crest, and hip circumference was measured over the widest part of the gluteal region.

Type 2 diabetes was diagnosed using the following WHO criteria: fasting blood glucose >6.7 mmol/l or 2-h blood glucose ≥10.0 mmol/l. Individuals lacking fasting blood glucose and OGTT data were considered affected if they were currently taking oral hypoglycemics and/or insulin. Deceased pedigree members were designated affected when clear documentation of type 2 diabetes diagnosis was available; they were otherwise treated as unknown. Type 1 diabetes was considered present if the patient had GAD antibodies, fasting C-peptide concentrations <0.3 nmol/l, or had required insulin treatment within 3 months of diabetes onset. Subjects so identified were not used for linkage analysis. To avoid families segregating MODY mutations, subjects with age of onset <35 years were excluded independent of their GAD antibody and C-peptide status. All pedigrees were subjected to preliminary forensic genotyping to exclude nonpaternities or adoptions. Pedigrees lacking a sib-pair affected with type 2 diabetes on the basis of the above exclusion criteria were not used for linkage analysis. We additionally excluded families in which both of the proband's parents had type 2 diabetes diagnoses.

Genotyping. Genomic DNA was extracted from whole blood using a commercially available kit (Gentra), then it was quantitated by Hoechst dye fluorescence, normalized at 40 ng/μl, and stored at 4°C. Microsatellite loci were selected from public databases to provide ~10 cM intermarker distances. Polymerase chain reaction primers labeled for detection by the ABI 377XL DNA sequencer (Applied Biosystems) were prepared using standard oligonucleotide synthesis chemistry. Each genotype was double-scored, once by an expert technician and once by a proprietary software package; incongruities between the two were resolved by the human scorer. Marker data for each pedigree were checked for Mendelian inheritance; raw data for all observed deviations were re-evaluated.

Linkage analysis. Our sample was stratified using three different subphenotypes: age at onset of type 2 diabetes (AGE), BMI, and ratio of waist circumference to hip circumference (WHR); recent work has shown that WHR exhibits greater heritability than many other measures of central obesity (M. Lehtovirta, unpublished data). BMI and WHR were age- and sex-corrected before calculation of pedigree means as follows: population means for both sexes within each decadal age class were estimated from phenotypes of unaffected individuals ($n = 529$). Pedigree means were then calculated using each affected individual's deviation from the appropriate sex and decadal mean. Corrected BMI and WHR were correlated in affected individuals (male subjects: $r = 0.45$, $P < 0.0001$; female subjects: $r = 0.32$, $P < 0.0001$) and in the entire sample (male subjects: $r = 0.45$, $P < 0.0001$; female subjects: $r = 0.42$, $P < 0.0001$). Neither variable was significantly correlated with age at onset in either the affected members or the entire sample ($r < 0.1$, $P > 0.05$). Data for BMI, WHR, and AGE are summarized in Table 1, as are threshold values of these variables for each subphenotype. As anticipated, there was substantial heterogeneity in the type 2 diabetes therapeutic modality used by subjects at the time of ascertainment: 21.5% used insulin, 43.7% used orally administered hypoglycemics, and 12.6% used a combination of the two; 18.8% reported using diet to control hyperglycemia; and 3.4% reported no therapeutic regimen.

Sex-averaged marker maps were determined by analysis of Centre d'Etude du Polymorphisme Humain (CEPH) pedigree data, using MultiMap (9); markers lacking CEPH data were placed using the integrated marker map maintained by the Genome Database (http://www.gdb.org). Multipoint nonparametric affected sib-pair (ASP) linkage analysis was performed using Genehunter version 1.0 (5) as modified by Kong and Cox (10). Allele frequencies were determined using actual or imputed founder genotypes.

We performed 4 analyses for each subphenotype, using the most extreme 20, 30, 40, and 50% of pedigrees as appropriate to the relevant variable (smaller values for AGE and larger ones for WHR and BMI), for a total of 12 subset analyses in addition to the original unstratified analysis. This approach differs from that taken by Mahtani et al. (4); their sample was subdivided into quartiles, and both the half and the quarter of families falling on each end of the phenotypic spectrum were analyzed separately. We did not use this strategy because we began the study design process with explicit hypotheses regarding the directionality of influence of the stratification phenotypes on severity of type 2 diabetes risk; specifically, we hypothesized that greater obesity values and earlier age at onset should identify those subjects with increased genetic loading for type 2 diabetes. Thus, we chose only to examine subsets taken from these tails of the phenotypic distributions; by examining four subsets from each, we performed the same number of tests as we would have performed using the quartiles strategy. Therefore, this strategy is equivalent to that of Mahtani et al. (4) from the perspective of multiple hypothesis testing.

Computer simulations. In light of the multiple testing inherent in the 12 semi-independent analyses described above, we conducted the following computer simulations to estimate the significance of our findings. These simulations provided an unbiased empirical P value for the following null hypothesis: the subset analysis performed on our actual observed data produced the results that we saw solely because of the increased opportunity to observe a high logarithm of odds (LOD) score afforded by multiple testing, and they were not attributable to an actual biological relationship between the phenotypic characteristics used to define the subsets and the chromosomal location at which linkage was observed. To address this question, we simulated our entire analysis 500 times and determined the proportion of these simulations that yielded a Genehunter NPL (nonparametric linkage)-all score, at any chromosomal location for any phenotype, in excess of our actual peak NPL-all score. In our actual analysis, we genome-scanned a total of 353 families before stratification. For each of the 13 analyses, Genehunter NPL-all scores, as well as Genehunter-Plus LOD scores, were recorded at increments of 2 cM across all 22 autosomes (all pedigrees weighted equally).

We conducted our simulations in adherence to the methods used for our actual analyses, conditioning on the NPL-all scores obtained from the Genehunter analysis of the actual data. Replicate samples were generated by taking the original set of 353 pedigrees and randomly assigning to them NPL-all vectors drawn with replacement from the set of observed NPL-all vectors. An NPL-all vector for a given pedigree was defined as the set of all NPL-all scores at 2-cM spacing across all autosomes for that pedigree. By sampling entire vectors, we were able to account for the between-locus correlation of NPL scores that exists in the actual data. Moreover, strata membership for pedigrees was held fixed to preserve observed between-strata correlations. For example, if a given pedigree in the actual sample was among the highest 40% of pedigrees for BMI and the highest 30% of pedigrees for waist/hip ratio, but was not among the earliest 50% for age of onset, then this was also true for each replicate; only the pedigree's NPL-all vector varied across replicates.

Finally, for each replicate, we computed the total NPL-all score for each of the 13 phenotypes at 2-cM increments across all autosomes, weighting all pedigrees equally, and recorded the highest NPL-all score obtained. We followed this procedure for each of the 500 replicates, obtaining an empirical distribution of genome-wide phenotype-wide maximum NPL-all scores, to which we compared our observed maximum NPL-all score.

In addition to producing an unbiased estimate of the empirical P value associated with the null hypothesis articulated above, this procedure also provides a conservative test of the more general null hypothesis that the highest LOD score resulting from our genome scan plus subset analyses is not greater than that which might be expected under the assumption that no disease susceptibility locus is present anywhere in the genome. This procedure is a conservative test of this hypothesis, owing to between-replicate correlation and to the fact that it conditions on the actual genome scan data, which will contain elevated NPL-all scores in some regions simply by chance. It is expected to be even more conservative when applied to data sets reflecting actual linkage to disease susceptibility loci.

RESULTS

Our genome-wide linkage analysis included 1,488 subjects—959 of whom were affected with type 2 diabetes—belonging to 353 pedigrees, and it provided a total of 480 ASPs. The difference between these numbers and the total number of families and subjects ascertained reflects exclusion of some families that did not meet the study criteria outlined in RESEARCH DESIGN AND METHODS. Owing to the late onset typical of type 2 diabetes, only 4 of the

pedigrees included DNA samples for both founders and 27 included samples for a single founder, leaving 322 with no parental genotype information. We genotyped 468 autosomal microsatellite markers, providing a mean intermarker interval of 8.8 cM. Mean heterozygosity was 0.76, and on average 96% of the subjects were successfully typed at each marker.

As anticipated in cases of substantial genetic heterogeneity, analysis of our entire unstratified sample resulted in only modest evidence for linkage; multipoint LOD scores >1.0 occurred on chromosomes 4 (LOD 1.41 near marker D4S2361) and 17 (LOD 1.29 near D17S1301). Stratification by AGE did not greatly increase evidence for linkage. Analysis of the earliest-onset 20% of our sample resulted in an LOD 2.41 near marker D5S816; no other age-based classification produced an LOD >2.0. Analysis of pedigrees falling in the upper 50% of WHR produced an LOD 2.38 near marker D1S3462; no other classification using this variable resulted in an LOD >2.0. LOD plots reflecting unstratified analyses of all chromosomes, the stratification results described above, and the marker maps used for these analyses are available on-line at www.diabetes.org/diabetes/appendix.asp.

Stratification by BMI proved more productive, increasing evidence for linkage to chromosome 18p from LOD 0.66 for the unstratified sample to LOD 4.22 (between D18S452 and D18S843) when the most obese 20% of pedigrees were analyzed. Figure 1 illustrates the pronounced effect of BMI stratification on this result; note that the LOD score increases with each successively more restrictive phenotypic definition, despite concomitant decreases in sample size. The class providing the greatest evidence for linkage contains 72 pedigrees and 78 ASP; the mean BMI of affected individuals within that group is 35.8 kg/m^2 vs. 29.1 kg/m^2 for all affected individuals and 26.8 kg/m^2 for the entire sample. No other BMI-based stratification resulted in a LOD >2.0, although the same 20th percentile stratum did slightly increase evidence for linkage to chromosome 17 (LOD 1.82).

Owing to the paucity of founder genotypes in our data set, overall information content was relatively low (0.425), as it was at the point of greatest evidence for linkage on 18p (0.472). Although our linkage analyses were corrected for the nonnormal null expectation in cases of incomplete information (10), we felt that additional marker data were required for a robust estimate of the actual degree of support. Accordingly, we typed 41 additional microsatellite markers spanning the short arm of chromosome 18, raising average information content across the linked region to 0.659 while reducing the average intermarker interval to 1.5 cM. Reanalysis using this denser marker map resulted in a maximum multipoint LOD of 3.79 (Fig. 2); we believe this slightly smaller value much more accurately reflects the degree of support provided by our sample for a type 2 diabetes susceptibility locus on 18p.

The significance of this LOD score, in light of multiple subset analyses, was evaluated using the simulation strategy previously described, wherein 136 of 500 replicates yielded a genome-wide phenotype-wide maximum NPL-all score that exceeded the observed maximum or occurred, on average, 0.272 (95% CI = 0.233–0.311) times per full genome scan using 12 phenotypic subsets. Thus, given our
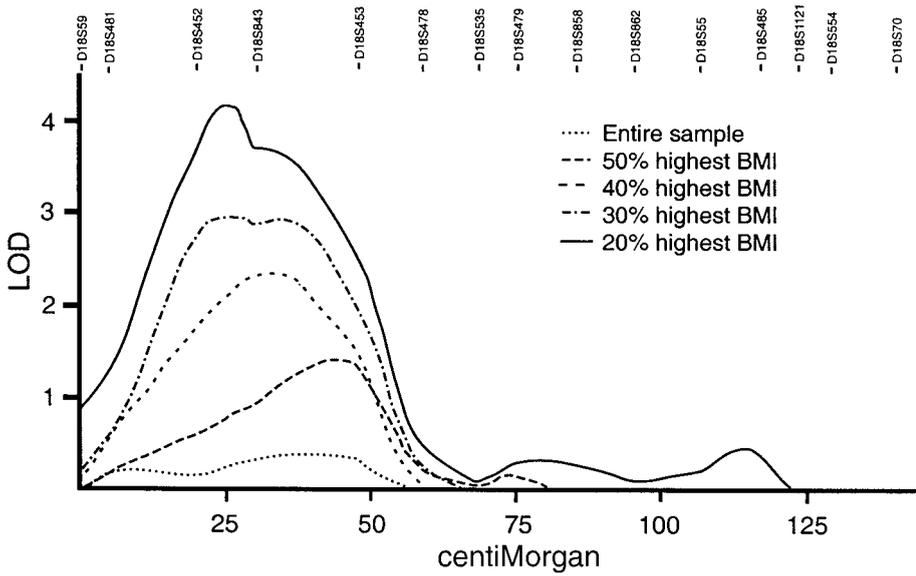
FIG. 1. Multipoint allele sharing analysis of the type 2 diabetes phenotype, stratified by pedigree mean age- and sex-adjusted BMI, using a 15-marker chromosome 18 framework map.

observed genome scan data, we cannot conclusively reject the null hypothesis that the observed improvement in the LOD score with subset analysis might be attributable only to multiple testing. As for its significance with respect to the null hypothesis (i.e., no disease susceptibility locus being present in the study sample), Lander and Kruglyak (11) defined a "suggestive" score as one that is expected, on average, to occur one time per full genome scan under the null hypothesis, and they defined a "significant" score as one that is expected, on average, to occur 0.05 times

(this null hypothesis states that no disease-predisposing gene exists anywhere in the genome). The results of our simulations (expected to be conservative for this null hypothesis, as detailed above) place our finding on chromosome 18p clearly between these thresholds, and therefore in the suggestive category.

DISCUSSION

We are unaware of any previous genome-wide analysis producing evidence for a type 2 diabetes susceptibility
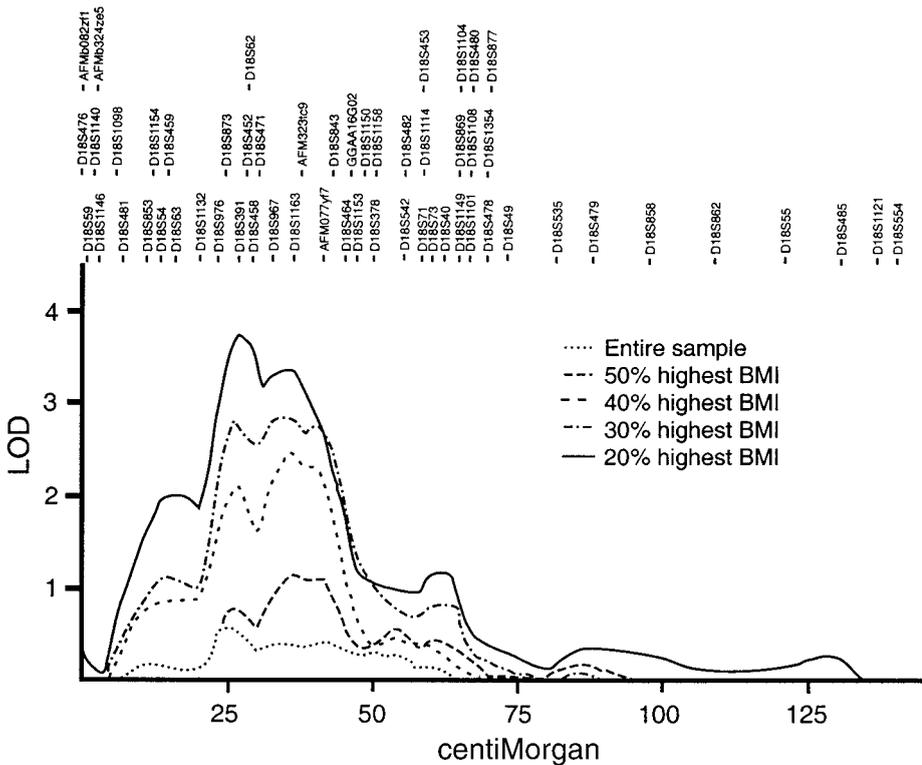

FIG. 2. Reanalysis of chromosome 18 using a 56-marker map.

gene on chromosome 18p, suggesting that our investigation has revealed the location of a gene not previously implicated in type 2 diabetes risk. Similarly, there have been no reported significant associations of chromosome 18p sequence polymorphisms with type 2 diabetes–related phenotypes. However, two recent studies have evaluated the role of chromosome 18 genes in human obesity. Chagnon et al. (12) detected both linkage and association of restriction fragment–length polymorphisms (RFLPs) in melanocortin receptor 5 (MC5R) to BMI, and they detected an association of a melanocortin receptor 4 (MC4R) RFLP with fat mass and percent body fat, but not with BMI. MC5R has been localized to chromosome 18p11.2 (13); this is centromeric of the point of maximum linkage in our study, but not so distant as to completely exclude potential identity. Ohman et al. (14) detected linkage to microsatellite markers flanking MC4R in nondiabetic obese sib-pairs. MC4R has been mapped (15) to 18q22, precluding identity with the chromosome 18p locus suggested by our results.

In addition to chromosome 18p, our investigation revealed moderate, albeit nonsignificant, evidence for linkage of type 2 diabetes–based phenotypes to regions of the genome implicated by other, similar studies, or to regions harboring genes thought to play a role in type 2 diabetes pathogenesis. Pratley et al. (16) observed linkage of fasting plasma insulin to chromosome 4p15-q12 in the Pima Indian population; the same interval was implicated in our initial unstratified analysis. Additionally, our observed linkage to chromosome 4, near marker D4S2361, colocalizes quite precisely with NKX6A, a homeobox gene that is expressed in islet β-cell lines and is suggested to play a role in islet development and/or regulation of insulin biosynthesis (17).

The glucagon receptor gene, GCGR, was mapped by two independent groups (18,19) to chromosome 17q25, near the place where we observed the greatest evidence for linkage to that chromosome (D17S1301). A missense mutation in that gene was shown to be associated with type 2 diabetes in a pooled French and Sardinian sample (20); the frequency of that same mutation was substantially elevated in hypertension patients compared with control subjects (21). Elbein et al. (22) demonstrated modest evidence for linkage of type 2 diabetes–based phenotypes to a polymorphism flanking the growth hormone variant GH2, ~20 cM centromeric of the point at which we observed maximum linkage to 17q. We are unaware of any reports of linkage or association of type 2 diabetes with markers near those where we observed linkage to chromosomes 1 and 5 (D1S3462 and D5S816).

On the whole, our findings are only moderately congruent with other type 2 diabetes linkage reports. Several studies have implicated chromosome 12q (8,23,24), whereas others have implicated chromosome 20q near marker D20S197 (23,25–28). We observed only modest evidence for linkage to 12q (LOD = 1.85 at marker D12S378 in the 30% highest BMI subset); even less support was available for linkage to chromosome 20 (LOD 0.70 at marker D20S891 in the 20% highest WHR subset). Other notable recent reports include linkage near the q telomere of chromosome 2 (29), where we observed no evidence for linkage using any phenotypic classification, and to chromosome 4q (28), where we observed LOD 1.18 at marker D4S2368 in the 30% earliest AGE subset. As noted above, the greatest evidence for linkage to chromosome 4 in our study occurred near marker D4S2361, which was >70 cM away from the Permutt et al. (28) result.

This absence of strong agreement among type 2 diabetes linkage studies is not unexpected, and it should be emphasized that even those loci enjoying apparent replication (12q, 20q) are implicated in only a minority of studies. Given that multiple physiological and developmental pathways (each populated by multiple genes) are likely to play a role in type 2 diabetes pathogenesis, coupled with the diverse population genetic histories of human ethnic groups, it seems that locus heterogeneity should be the rule, not the exception, when different populations are investigated. The observation that repeated studies of the same population (e.g., Finns) may also implicate different regions of the genome underscores the importance of microgeographic differentiation, allelic heterogeneity, and sampling variance in studies of complex trait genetics.

The implications are as yet unclear regarding the observed relationship between BMI and linkage for the pathophysiological role of the chromosome 18p type 2 diabetes susceptibility locus suggested by our results, principally because the relationship between diabetes and obesity is incompletely understood. It is often suggested that obesity plays a causal role in development of type 2 diabetes (30), owing to the peripheral insulin resistance characteristic of type 2 diabetes, the clear relationship between obesity and insulin resistance (31), and the observation that weight loss typically improves insulin sensitivity in type 2 diabetes patients (32). Additional support for this hypothesis is found in the recent demonstration of significantly increased β-cell apoptosis in obese versus lean ZDF rats (33). In this context, the chromosome 18p type 2 diabetes susceptibility locus suggested by our results might be interpreted as a modifier of the degree of diabetes risk conferred by obesity, or simply as an obesity predisposition locus that contributes in this fashion alone to type 2 diabetes risk.

The obesity-as-risk-factor hypothesis is called into question, however, by the observation that the large majority of obese individuals are as comparably insulin resistant as type 2 diabetes patients but simultaneously normoglycemic (31) as well as by longitudinal studies showing that impaired insulin sensitivity, detected before diabetes onset and independent of obesity, is a robust predictor of eventual progression to type 2 diabetes (34,35). Thus, as an alternative hypothesis, we might imagine two (or more) independent etiological pathways, both with fasting hyperglycemia as their end point, one of which also leads to increased risk of obesity. In this scenario, subphenotyping using BMI should increase genetic homogeneity and thus evidence for linkage (as we observed), but obesity would correctly be interpreted as a correlate, not a cause, of progression to type 2 diabetes. To resolve this issue, substantial additional research will be required, including both molecular characterization of the chromosome 18p susceptibility locus suggested by our results and ascertainment of a sample of obese nondiabetic sib-pairs from the Scandinavian population.

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