Examination of Candidate Chromosomal Regions for Type 2 Diabetes Reveals a Susceptibility Locus on Human Chromosome 8p23.1

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In a panel of large Caucasian pedigrees, we genotyped markers in eight chromosomal regions previously reported as supporting linkage with type 2 diabetes. We previously reported significant linkage on chromosome 20q (maximum logarithm of odds score [MLS] = 2.79) in this panel. In the present analysis, candidate regions on 1q, 2q, 3q, 5q, 9q, and 10q yielded little evidence for linkage; a region on 2p (MLS = 1.64, $P = 0.01$ at position 9.0 cM) gave suggestive evidence of linkage; and a region on 8p (MLS = 3.67, $P = 2.8 \times 10^{-5}$, at position 7.6 cM) gave significant evidence of linkage. Conditional analyses were performed for both 2p and 8p regions and the region reported on 20q. The MLS for 2p increased from 1.64 to 1.79 (empirical $P = 0.142$) when conditioned for heterogeneity on 20q. The case was similar for 8p, where the MLS increased from 3.67 to 4.51 (empirical $P = 0.023$) when conditioned on families without evidence of linkage at 20q. In conclusion, our data support a type 2 diabetes susceptibility locus on chromosome 8p that appears to be independent from other susceptibility loci. Although we were able to replicate linkage in our pedigrees on chromosome 2p, we did not find evidence of linkage for regions on 1q, 2q, 3q, 5q, 9q, or 10q. *Diabetes* 53:486–491, 2004

Type 2 diabetes is a disease characterized by hyperglycemia, insulin resistance, and impaired insulin secretion (1). Although the pathophysiology of this disease is not well understood, evidence of familial clustering indicates that genetic susceptibility plays a role in disease development (2,3). Identification of genetic susceptibility factors, however, has been difficult, and, to date, the putative genes contributing to the etiology of type 2 diabetes remain unknown.

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IGT, impaired glucose tolerance; LOD, logarithm of odds; MLS, maximum LOD score; PPase, protein phosphatase.

Efforts to identify the genetic factors underlying susceptibility to type 2 diabetes have used both candidate gene approaches and linkage studies (4). More than a dozen genomewide scans have been completed from which various chromosomal regions with suggestive or significant evidence of linkage with type 2 diabetes have emerged (5–23). However, despite intense efforts to clone the susceptibility genes, the identification of these genes has been unsuccessful, with the single exception of calpain-10 (*CAPN10*) or *NIDDM1* (24).

Efforts to clone type 2 diabetes susceptibility loci have been plagued by inconsistent findings. Evidence for linkage with type 2 diabetes has been reported on most autosomes (25). However, there has been little overlap among linkage results, with the notable exceptions being the findings on chromosomes 1q (8,11,15,19,23) and 20q (26–30). Factors contributing to the failure to replicate linkage results include the presence of environmentally determined phenocopies of type 2 diabetes as well as the complex nature of the inheritance of type 2 diabetes. The latter includes genetic and allelic heterogeneity, epistasis (gene-to-gene interaction), and gene-to-environment interactions.

In a recent study, we showed that the overall sibling genetic risk ratio (λ_S) for type 2 diabetes may be less than previously assumed (2,3) and is similar to that found in the general population. Moreover, the observed risk was comparable in siblings of index cases without a history of parental diabetes (>50% of all diabetic patients), suggesting a smaller overall genetic contribution to the diabetic etiology in these patients (3). In contrast, the sibling recurrence risk of diabetes in families having a diabetic parent was elevated ($\lambda_S = 2.0$). Furthermore, in a substantial proportion of these families, diabetes was transmitted vertically through three generations. A $\lambda_S = 3.5$ in this subset indicates a significantly higher risk to siblings. By studying families with a type 2 diabetes inheritance pattern consistent with a single, highly penetrant, dominant susceptibility locus, type 2 diabetes gene mapping may be achievable using classic linkage methods. Recently, we demonstrated the feasibility of this approach by collecting a panel of large families among whom type 2 diabetes was transmitted vertically. A putative locus for type 2 diabetes was identified on chromosome 20q13 using a panel of highly polymorphic microsatellite markers. Positive evidence for linkage was found for a 10-cM region between markers D20S119 and D20S428, with the strongest evi-

dence occurring at D20S196 (MLS = 2.79) (26,30). Using this approach of ascertaining highly informative (and more genetically homogeneous) families, it is anticipated that positional cloning of the putative susceptibility genes for type 2 diabetes will be achieved.

To further exploit this general approach and gain a better understanding of the inheritance of type 2 diabetes, chromosomal regions previously identified in families of European origin were investigated in our family collection. Criteria for selecting the chromosomal regions to be explored included suggestive evidence for linkage (MLS $\geq$1.5) in other studies (6,11–13,15,19,21,22). A subset of regions meeting these criteria were selected and subsequently examined in our panel of 43 large families with type 2 diabetes. A total of eight candidate chromosomal regions were considered in the present analysis: 1q, 2p, 2q, 3q, 5q, 8p, 9q, and 10q.

RESEARCH DESIGN AND METHODS

Families for the Joslin Study on the Genetics of Type 2 Diabetes were ascertained through index cases from the Joslin Clinic population; details of their recruitment have been previously described (26,30,31). Briefly, we sought to identify families with a pattern of occurrence of type 2 diabetes that was consistent with an autosomal-dominant mode of inheritance. The criteria used to identify eligible families consisted of *1*) an index case and at least one sibling having been diagnosed with type 2 diabetes between ages 35 and 59 years, *2*) the index case having been treated for at least 2 years with diet or oral agents, and 3) the occurrence of diabetes in at least three generations within each family.

All study participants completed medical and family history questionnaires and had fasting blood samples drawn for blood glucose determination, DNA extraction, and other biochemical measurements. All nondiabetic and diabetic individuals treated with oral agents or diet had blood samples drawn 2 h after a 75-g oral glucose challenge. Glucose levels were measured at the Joslin Clinic laboratory using a Synchron CX Delta system (Beckman Coulter, Fullerton, CA) or One-Touch home glucose meters (Lifescan, Newtown, PA). The latter measurements were re-expressed in terms of venous plasma glucose as measured in the Joslin Clinic laboratory. Serum insulin was measured by radioimmunoassay by Linco Research (St. Charles, MO). In addition, measurements of height, weight, and blood pressure were taken. Diabetes and impaired glucose tolerance (IGT) status were diagnosed according to World Health Organization criteria (32).

A total of 534 individuals, including 247 with type 2 diabetes and 30 with IGT, from a collection of 43 families of European origin were included in the present study. This was the same panel of families studied by Klupa et al. (30). A summary of these pedigrees and the number and type of affected relative pairs can be viewed in online appendix A at http://diabetes.diabetesjournals.org. Data for this appendix were compiled using the MEGA2 version 2.3 software program (33). An average of 12.4 individuals (6.3 with type 2 diabetes and 6.1 with normal glucose tolerance) were examined from each family. The mean age at diagnosis of diabetes was 47 $\pm$ 17 years. The mean age at enrollment into the study was 59 $\pm$ 14 years for individuals with diabetes and 49 $\pm$ 18 years for individuals with normal glucose tolerance.

The Committee on Human Subjects of the Joslin Diabetes Clinic approved the study protocol and informed consent procedures.

Microsatellite genotyping. All individuals were genotyped at each of the eight candidate chromosomal regions using a total of 48 highly polymorphic microsatellite markers. The names and descriptions of all markers used can be seen in online appendix B. Marker positions were based on the Marshfield sex-averaged genetic map (http://research.marshfieldclinic.org/genetics/). Markers MS1, MS2, and MS3, novel microsatellites developed for fine mapping on chromosome 8p, were positioned based on the physical map from Build 33 of the National Center of Biotechnology Information human genome assembly (http://www.ncbi.nlm.nih.gov). The average marker heterozygosity was 0.79, with a range of 0.66–0.94. The average spacing between adjacent markers was 7.3 cM. Fluorescence-based genotyping was performed for all markers using primer pairs in which all forward primers were labeled at their 5′ end with TET, FAM, or HEX fluorescent dyes (MWG Research, High Point, NC/Research Genetics, Huntsville, AL). PCRs were performed in a 15-μl volume reaction in a 96-well PCR plate (Greiner Labortechnik, Gloucestershire, U.K.). Each reaction contained 4 ng genomic DNA, 4 pmol of each primer, 25 μmol/l dNTPs (Amresco, Solon, OH), 1–2 mmol $MgCl_2$, 3 μl of 5 × PCR buffer, and 0.2 units of Amplitaq Polymerase (Applied Biosystems, Foster City, CA). The PCR program for all markers was as follows: 5 min denaturation at 95°C, held at 80°C for the addition of *Taq*, 35–40 cycles of 95°C for 30 s, 50–59°C for 30 s, and 72°C for 30 s, then a 10-min extension at 72°C. All reactions were amplified using PTC-100 thermocyclers (MJ Research, Watertown, MA) and subsequently electrophoresed using an ABI Prism Model 377 DNA Sequencer (Applied Biosystems). All genotype data were analyzed by two different observers using both GENESCAN 3.12 (Applied Biosystems) and GENOTYPER 2.5 (Applied Biosystems) software.

Statistical analysis

Linkage analysis. Marker allele frequencies were estimated using standard gene counting and all available unrelated pedigree founders, with 95% complete genotypic data available. Because of the likelihood that delayed penetrance among unaffected individuals might result in their misclassification, as well as residual uncertainty about the mode of inheritance of type 2 diabetes, a nonparametric linkage analysis method was chosen. These analyses were performed using the GENEHUNTER-PLUS version 1.2 (34) computer program (scoring function = S_{all}) followed by estimation of allele sharing−model logarithm of odds (LOD) scores and likelihood ratio *Z* scores (Z_{lr}) using ASM (35), an ancillary software program that allows for individual weights to be assigned to each family and relies on output files from GENEHUNTER-PLUS to calculate the associated Z_{lr} and LOD scores. Allele sharing-model LOD scores were calculated under the null hypothesis of no linkage using an exponential model with δ constrained between −1 and 1 at each of the eight candidate chromosomal regions and with equal weight assigned to all 43 families (35). For the purpose of our linkage analysis, individuals with IGT (n = 30) and normal glucose tolerance (n = 257) were used only to establish allele sharing; that is, their affection status was not considered in the analysis.

Conditional analysis. To examine potential epistatic/heterogeneic effects among the candidate chromosomal regions linked with type 2 diabetes, conditional analyses were performed using the ASM program for chromosomal regions showing evidence of linkage, including our previously reported linkage evidence on chromosome 20q (30). To model both epistasis (consistent with gene-to-gene interaction) and heterogeneity (consistent with additivity), a series of analyses was performed in which each family was assigned a "weight," either 0 or 1, for each region based on the evidence for linkage as reflected by the maximum multipoint Z_{lr} in that region. To model epistasis, a weight of 1 was assigned to families with a maximum multipoint $Z_{lr} > 0$ at that location, with a corresponding weight of 0 being assigned to all others. Similarly, heterogeneity was modeled by assigning a weight of 0 to families with a maximum multipoint $Z_{lr} > 0$ at that location, with a weight of 1 assigned to all other families. Assigning weights of 0 to families effectively excluded them from the analysis. Only families with any positive support for linkage (i.e., those receiving a weight of 1) contributed to the resulting LOD scores.

To address the possibility that any observed significance based on our partitioning may be due to the information content of the families included in the analyses, a simulation approach to determine the "empirical" significance, regardless of the families included in the analyses, was used. Significance of the conditional MLS was evaluated by computer-simulated permutation testing. To maintain the proportion of families with evidence of linkage from the unweighted analysis (while allowing any family to be considered "supporting" linkage), each simulation randomly assigned a weight of 1 to a subset of families. This new distribution of families supporting linkage was used to recalculate the LOD score. The procedure was repeated 10,000 times, allowing the empirical P value to be estimated from the proportion of computer-generated replicates that exceeded the observed conditional MLS score.

RESULTS

Two-point and multipoint linkage results obtained from analysis of the eight candidate chromosomal regions in all 43 families are summarized in Table 1. We were unable to replicate previously reported linkage of type 2 diabetes with markers on 1q, 2q, 3q, 5q, 9q, and 10q. However, some evidence for linkage was observed on 2p; the two-point linkage analysis gave MLS = 1.95 at marker D2S2166. This evidence was slightly reduced in multipoint analysis (MLS = 1.64, P = 0.01, at position 9.0 cM). The strongest evidence for linkage was found on chromosome 8p; the two-point linkage analysis yielded MLS = 1.72 (P = 0.05) for marker D8S1819 located at the telomeric boundary of the examined region. This evidence significantly increased in multipoint analysis (MLS = 3.62, $P = 1.8 \times 10^{-4}$, at

TABLE 1
Two-point and multipoint linkage analysis results for eight candidate chromosomal regions

Chromosome	Examined interval (cM)	Two-point $MLS_{(cM)}$	δ	*P*	Nearest marker	Multipoint $MLS_{(cM)}$	δ	*P*	Nearest marker
1q	168–252	$0.78_{(205)}$	0.35	0.31	D1S1189	$0.05_{(205)}$	0.09	0.17	D1S1189
2p	1–20	$1.95_{(9)}$	0.32	0.01	D2S2166	$1.64_{(9)}$	0.29	0.01	GATA158C03/D2S2166
2q	260–263	$0.45_{(260)}$	0.24	0.14	D2S125	$0.006_{(263)}$	−0.04	0.52	D2S140
3q	201–214	$0.31_{(207)}$	0.19	0.18	D3S1580	$0.004_{(207)}$	0.03	0.42	D3S1580
5q	70–85	$0.15_{(85)}$	−0.21	0.70	D5S1397	$0.19_{(85)}$	−0.20	0.76	D5S1397
8p	9–46	$1.72_{(9)}$	0.34	0.05	D8S1819	$3.62_{(9)}$	0.35	0.00018	D8S1819
9q	51–84	$0.64_{(51)}$	0.34	0.25	D9S1875	$0.09_{(66)}$	0.10	0.27	D9S301
10q	75–156	$0.002_{(128)}$	0.02	0.44	D10S1750	$0.14_{(128)}$	−0.20	0.71	D10S1750

For two-point and multipoint MLS, value in parentheses indicates position in cM from *p*-telomere.

position 9.0 cM). To examine this result further, we genotyped our families for eight additional microsatellite markers located in the *p*-telomeric region (0–10 cM). The linkage peak shifted to a more telomeric position (D8S1742, at position 7.6 cM) with the two-point MLS increasing to 3.08 ($P = 4.0 \times 10^{-3}$) and the multipoint MLS increasing to 3.67 ($P = 2.8 \times 10^{-5}$) (Fig. 1).

In all, 8 of the 43 families had an estimated MLS ≥0.5 at marker D8S1742. One family with nine affected individuals in three generations, all sharing the same haplotype, contributed the greatest support for linkage. After removal of this family, the evidence for linkage in the seven remaining families was still significant (MLS = 2.23, $P = 8.4 \times 10^{-4}$), with the MLS occurring at the same chromosomal location.

To examine epistatic and heterogeneity effects among the chromosomal regions linked with type 2 diabetes, we performed conditional analyses using the results for 2p and 8p and the previously reported results for 20q (30) (Table 2). The Z_{lr} scores for each pedigree at the markers used for these analyses and a summary of families receiving weights of 1 and 0 under each model can be viewed in online appendix C. For each region, the multipoint MLS for the total family collection is provided along with the MLS for the family subsets. The MLS for the region on chromosome 2p decreased from 1.64 in all families to 0.72 when only families with positive Z_{lr} scores on 8p were examined; this finding supported the absence of an epistatic effect between the two chromosomal regions. Similarly, we found a decrease in MLS when linkage with 2p was analyzed in a subset of families with negative Z_{lr} scores on 8p, suggesting the absence of heterogeneity between these two regions. The results of the conditional analyses between 2p and 20q differed. Whereas the MLS for 2p under an epistatic model decreased significantly (almost to zero), it increased (empirical $P = 0.142$) above the MLS in all families when the analysis was carried out only in families with negative Z_{lr} scores on 20q (from 1.64 to 1.79).

A similar trend was observed when a conditional analysis was performed for chromosome 8p; however, the lack of an epistatic effect and the presence of a heterogeneity effect were much more pronounced (Table 2). Evidence of epistatic and heterogeneity effects between 8p and 2p were not significant. The MLS for 8p conditioned under an epistatic model with evidence at 20q decreased to 0.15 from 3.67. Significant evidence of heterogeneity (empirical $P < 0.023$) was observed between 8p and 20q; that is, evidence for linkage with the former region was particularly strong in families that did not show evidence for linkage with chromosome 20q. The MLS increased from 3.67 in all families to 4.51 in a subset of families not linked with 20q.

The results from the conditional analysis (heterogeneity model) for chromosomal regions on 8p and 20q are shown in Fig. 1. MLS statistics obtained in all 43 families for chromosome 8p are plotted over the 0–45 cM interval. The MLS is 3.67 at marker D8S1742, with a 10-cM support interval between markers D8S518 and D8S351. When families with negative Z_{lr} scores on chromosome 20 were removed from this analysis, the MLS for the remaining families increased to 4.51 ($P = 1.5 \times 10^{-5}$). The maximum MLS was observed at the same marker in the two analyses; however, the support interval was markedly reduced to ~3.5 cM (D8S518 to D8S277) when the families supporting apparent heterogeneity were removed.

Table 3 compares clinical characteristics of the 55 diabetic individuals in the families linked with 8p with those of the 214 diabetic individuals in the remaining 37 unlinked families. The age at diagnosis was similar for affected diabetic members of the families linked to chromosome 8p and diabetic members of the unlinked fami-

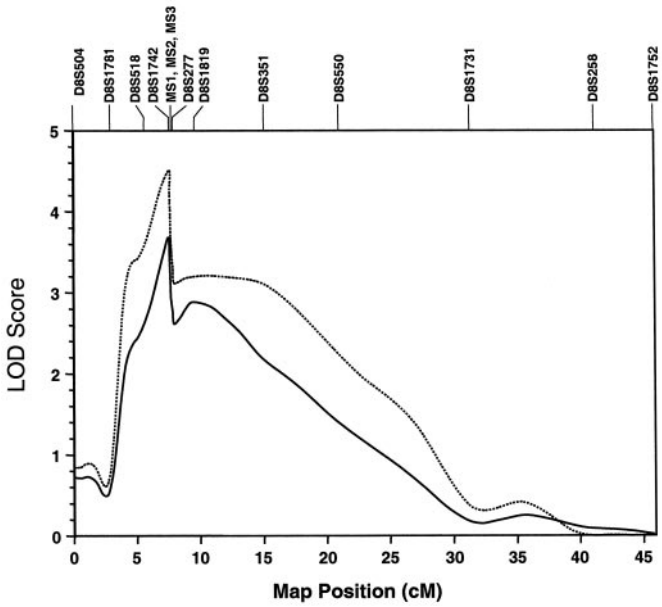

FIG. 1. Results from multipoint linkage analysis. Allele-sharing LOD scores are shown for chromosome 8p for all 43 families (——) and for families unlinked to chromosome 20q (----)

TABLE 2
Results of conditional multipoint linkage analysis

Chromosomal region	Region and specific conditional marker	MLS		
		All families	Linked families at specific marker (epistasis)	Nonlinked families at specific marker (heterogeneity)
2p25 at D2S2166	8p23 at D8S1742	1.64	0.72	0.91
	20q13 at D20S196	1.64	0.20	1.79*
8p23 at D8S1742	2p25 at D2S2166	3.67	3.39	0.47
	20q13 at D20S196	3.67	0.15	4.51†

*Empirical $P \leq 0.15$; †empirical $P \leq 0.05$. Empirical P values were estimated based on the number of computer-generated replicates that exceeded the observed conditional MLS score.

lies. The affected individuals from the linked families appear to be leaner, and fewer were treated with insulin compared with the unlinked families. Affected members from the linked families also had higher fasting glucose and lower 2-h serum insulin levels. When we controlled for fasting plasma glucose values using multiple regression analysis, this difference (between diabetic family members linked and not linked with 8p) disappeared.

DISCUSSION

Selecting families through type 2 diabetes index cases and when they have a history of vertical transmission of diabetes through an affected parent has the possible benefit of reducing genetic and phenotypic heterogeneity. Reducing the variation while increasing the genetic effect may increase statistical power and facilitate the search for type 2 diabetes susceptibility genes (3). Previously, this study design has had success in identifying genes responsible for maturity-onset diabetes of the young (36). Applying a similar approach to the search for genes contributing to a form of type 2 diabetes that is more typical, yet with enrichment of genetic effects, should result in the identification of susceptibility genes that may lie in more important physiological pathways.

We have previously demonstrated that this approach can be profitable by identifying a putative locus for type 2 diabetes on chromosome 20q using a collection of large families with vertical transmission of disease (26,30). To exploit this approach further, we used our families to replicate evidence for linkage reported previously by other authors. Eight candidate chromosomal regions met our criteria and were chosen for examination in our panel of 43 large families with type 2 diabetes segregating as autosomal dominant disorder.

Our most significant finding was observed on chromosome 8p. We identified a major susceptibility locus while attempting to replicate initial findings from a genome scan reported by Wiltshire et al. in the U.K. (19). However, we identified a possible type 2 diabetes susceptibility region more telomeric to the original report, with a support interval of ~10 cM, between D8S518 and D8S351. The evidence for linkage reported in the U.K. genome scan, MLS = 2.55 at position 42.2 cM, is very broad and extends into the region observed in our families. In addition, Elbein et al. (11) reported modest evidence for linkage of type 2 diabetes to chromosome 8p. In multigenerational families of European ancestry, suggestive evidence for linkage was observed at marker D8S136 (LOD score = 1.35 at position 44 cM) and D8S87/D8S532 (LOD score = 1.36 at position 61–65 cM).

Presently, one potential candidate gene for type 2 diabetes has been identified in our support interval: protein

TABLE 3
Clinical characteristics of diabetic members from families linked and unlinked to chromosome 8p

	Linked families	Unlinked Families	*P*
Number of families	8	35	—
Number of diabetic subjects (M/F)	28/27	109/105	—
Age at diagnosis (years)	50 ± 13	46 ± 15	0.06
Age at examination (years)	60 ± 12	59 ± 14	0.33
Percent IBW at examination	134 ± 23	140 ± 34	0.11
Lifetime maximum percent IBW	148 ± 28	156 ± 41	0.09
Systolic blood pressure	137 ± 17	136 ± 20	0.77
Diastolic blood pressure	79 ± 11	79 ± 10	0.98
Albumin/creatinine ratio	653 ± 1,902	475 ± 1,411	0.45
Treatment at examination (%)			
Diet alone	20.0	17.3	0.24
Oral agents only	47.3	37.4	
Insulin	32.7	45.3	
Non–insulin-treated diabetic patients			
Fasting blood glucose (mg/dl)	197 ± 80	156 ± 61	0.008
2-h blood glucose (mg/dl)	351 ± 90	270 ± 80	0.005
Fasting serum insulin (μU/ml)	12.4 ± 7.4	16.7 ± 12.9	0.12
2-h serum insulin (μU/ml)	22.4 ± 15.4	52.2 ± 65.7	0.01

Data are percent or means ± SD, unless otherwise indicated. IBW, ideal body weight.

phosphatase 1 regulatory (inhibitor) subunit 3 (*PPP1R3B*) (37). Because serine/threonine protein phosphatase (PPase) inactivation promotes insulin exocytosis in the β-cell, alterations in this PPase inhibitor may influence glucose-sensitive insulin secretion (38). This gene is being examined for DNA sequence differences in our linked families. The other transcripts in our support interval on 8p remain incompletely characterized, so other positional candidate genes may exist and yet (at this point) remain to be examined.

The clinical features of diabetic patients from our 8p-linked families were similar to those of diabetic patients from families not supporting evidence for linkage to this region. This suggests that a defect(s) in the putative susceptibility gene on 8p results in a phenotype consistent with the common insulin-resistant form of type 2 diabetes. Families from our collection that have been linked with chromosome 20q share this same phenotype.

Modeling the complex pattern of inheritance of type 2 diabetes, as well as other genetically complex traits, is challenging. Genetic and allelic heterogeneity, epistasis, and environmental factors each interact to confer disease susceptibility. To improve the ability to map genes in such complex disorders, two-locus linkage analyses have been proposed (39,40). Using the approach described by Cox et al. (41), we investigated these mechanisms for loci where evidence of linkage was observed. Our most significant observation was that of heterogeneity among loci located on chromosomes 8p and 20q (empirical $P < 0.023$). Interpreting the significance of this interaction, however, requires caution. Although our model approach attempted to minimize spurious observations, this possibility remains.

We observed suggestive linkage to type 2 diabetes on chromosome 2p (marker D2S2166, MLS = 1.64, at position 9.0 cM). In this same region, MLS = 0.87 at 5.5 cM, modest evidence for linkage was reported by Ghosh et al. in a Finnish-U.S. study (13). Moreover, in the same study, the investigators examined evidence for linkage on chromosome 20q in sib-pairs conditional with evidence of linkage on chromosome 2p (epistatic effect). The MLS on 20q increased from 2.15 to 5.06. Repetition of this conditional analysis based on linkage at a locus closer to our locus (D2S2166) increased the MLS further. In contrast to these findings, we were unable to replicate evidence for epistasis upon repeating this two-locus analysis in our dataset.

Similar interactions among loci associated with type 2 diabetes have previously been reported. Cox et al. (41) identified an epistatic interaction between the *NIDDM1* region of chromosome 2 and the *CYP19* region of chromosome 15. Wilshire et al. (19) have also shown evidence of epistasis at loci on 1q24.2 and 10q23.3 and heterogeneity between loci on 1q24.2 and 5q32.

Evidence of a type 2 diabetes susceptibility locus residing on chromosome 1q has been reported by several groups. Elbein et al. (11) reported significant evidence for linkage on chromosome 1q between markers CRP and APOA2 in a study of 19 multigenerational families. Others (15,19,22,23) have since replicated this evidence in their respective populations. In addition, significant evidence for linkage to this region was reported from the Pima Indian genome scan (8). All six reports have identified linkage signals across a region spanning ~25 cM, from positions 163 cM to 189 cM on chromosome 1q. We observed little evidence for linkage to this region in our families (MLS = 0.05, at position 205 cM).

Several additional regions were examined and, as was the case with chromosome 1q, we were unable to replicate evidence for linkage in this dataset. These chromosomal regions included 2q (260–263 cM), 3q (201–214 cM), 5q (70–85 cM), 9q (51–84 cM), and 10q (75–156 cM) (6,12,15,19,21,22). The possibility cannot be dismissed that genes in these regions may contribute to disease susceptibility in our families; however, these contributions are likely to be modest. Given the different evolutionary histories of the multiple family collections, it is not expected that all type 2 diabetes susceptibility loci will be equivalently important in all populations. Because our family collection favored an autosomal dominant mode of inheritance, all gene effects may not be represented in these pedigrees. Our data indicate that genetic defects at two major loci, located on chromosomes 8p23.1 and 20q13.1, act independently to confer susceptibility to type 2 diabetes. These two major loci accounted for susceptibility to diabetes in almost 40% of the families in our collection. The major locus/loci responsible for diabetes in the remaining 60% of our families has yet to be identified. Through continued research efforts, including a genome-wide screen currently under way, we hope to be able to provide further insight toward understanding the genetic basis of disease in these families.

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REFERENCES

1. Kahn CR: Banting Lecture: Insulin action, diabetogenes, and the cause of type 2 diabetes. *Diabetes* 43:1066–1084, 1994
2. Rich SS: Mapping genes in diabetes: genetic epidemiological perspective. *Diabetes* 39:1315–1319, 1990
3. Weijnen CF, Rich SS, Meigs JB, Krolewski AS, Warram JH: Risk of diabetes in siblings of index cases with type 2 diabetes: implications for genetic studies. *Diabet Med* 19:41–50, 2002
4. Stern MP: The search for type 2 diabetes susceptibility genes using whole-genome scans: an epidemiologist's perspective. *Diabetes Metab Res Rev* 18:106–113, 2002
5. Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B, Morrison VA, Wapelhorst B, Spielman RS, Gogolin-Ewens KJ, Shepard JM, Williams SR, Risch N, Hinds D, Iwasaki N, Ogata M, Omori Y, Petzold C, Rietzch H, Schroder HE, Schulze J, Cox NJ, Menzel S, Boriraj VV, Chen X, et al: A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nat Genet* 13:161–166, 1996
6. Mahtani MM, Widen E, Lehto M, Thomas J, McCarthy M, Brayer J, Bryant B, Chan G, Daly M, Forsblom C, Kanninen T, Kirby A, Kruglyak L, Munnelly K, Parkkonen M, Reeve-Daly MP, Weaver A, Brettin T, Duyk G, Lander ES, Groop LC: Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. *Nat Genet* 14:90–94, 1996
7. Pratley RE, Thompson DB, Prochazka M, Baier L, Mott D, Ravussin E, Sakul H, Ehm MG, Burns DK, Foroud T, Garvey WT, Hanson RL, Knowler

WC, Bennett PH, Bogardus C: An autosomal genomic scan for loci linked to prediabetic phenotypes in Pima Indians. *J Clin Invest* 101:1757–1764, 1998
8. Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennett PH, Knowler WC: An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet* 63:1130–1138, 1998
9. Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ, O'Connell P, Stern MP: Linkage of type 2 diabetes mellitus and age at onset to a genetic location on chromosome 10q in Mexican Americans. *Am J Hum Genet* 64:1127–1140, 1999
10. Hegele RA, Sun F, Harris SB, Anderson C, Hanley AJ, Zinman B: Genome-wide scanning for type 2 diabetes susceptibility in Canadian Oji-Cree, using 190 microsatellite markers. *J Hum Genet* 44:10–14, 1999
11. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ: A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes* 48:1175–1182, 1999
12. Ehm MG, Karnoub MC, Sakul H, Gottschalk K, Holt DC, Weber JL, Vaske D, Briley D, Briley L, Kopf J, McMillen P, Nguyen Q, Reisman M, Lai EH, Joslyn G, Shepherd NS, Bell C, Wagner MJ, Burns DK: Genomewide search for type 2 diabetes susceptibility genes in four American populations. *Am J Hum Genet* 66:1871–1881, 2000
13. Ghosh S, Watanabe RM, Valle TT, Hauser ER, Magnuson VL, Langefeld CD, Ally DS, Mohlke KL, Silander K, Kohtamaki K, Chines P, Balow JJ, Birznieks G, Chang J, Eldridge W, Erdos MR, Karanjawala ZE, Knapp JI, Kudelko K, Martin C, Morales-Mena A, Musick A, Musick T, Pfahl C, Porter R, Rayman JB: The Finland-United States investigation of non-insulin-dependent diabetes mellitus genetics (FUSION) study. I. An autosomal genome scan for genes that predispose to type 2 diabetes. *Am J Hum Genet* 67:1174–1185, 2000
14. Watanabe RM, Ghosh S, Langefeld CD, Valle TT, Hauser ER, Magnuson VL, Mohlke KL, Silander K, Ally DS, Chines P, Blaschak-Harvan J, Douglas JA, Duren WL, Epstein MP, Fingerlin TE, Kaleta HS, Lange EM, Li C, McEachin RC, Stringham HM, Trager E, White PP, Balow J Jr, Birznieks G, Chang J, Eldridge W: The Finland-United States investigation of non-insulin-dependent diabetes mellitus genetics (FUSION) study. II. An autosomal genome scan for diabetes-related quantitative-trait loci. *Am J Hum Genet* 67:1186–1200, 2000
15. Vionnet N, Hani E, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P: Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21–q24. *Am J Hum Genet* 67:1470–1480, 2000
16. Parker A, Meyer J, Lewitzky S, Rennich JS, Chan G, Thomas JD, Orho-Melander M, Lehtovirta M, Forsblom C, Hyrkko A, Carlsson M, Lindgren C, Groop LC: A gene conferring susceptibility to type 2 diabetes in conjunction with obesity is located on chromosome 18p11. *Diabetes* 50:675–680, 2001
17. Permutt MA, Wasson JC, Suarez BK, Lin J, Thomas J, Meyer J, Lewitzky S, Rennich JS, Parker A, DuPrat L, Maruti S, Chayen S, Glaser B: A genome scan for type 2 diabetes susceptibility loci in a genetically isolated population. *Diabetes* 50:681–685, 2001
18. Luo TH, Zhao Y, Li G, Yuan WT, Zhao JJ, Chen JL, Huang W, Luo M: A genome-wide search for type II diabetes susceptibility genes in Chinese Hans. *Diabetologia* 44:501–506, 2001
19. Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, Sampson M, O'Rahilly S, Frayling TM, Bell JI, Lathrop GM, Bennett A, Dhillon R, Fletcher C, Groves CJ, Jones E, Prestwich P, Simecek N, Rao PV, Wishart M, Bottazzo GF, Foxon R, Howell S, Smedley D, Cardon LR, Menzel S, McCarthy MI: A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. *Am J Hum Genet* 69:553–569, 2001
20. Busfield F, Duffy DL, Kesting JB, Walker SM, Lovelock PK, Good D, Tate H, Watego D, Marczak M, Hayman N, Shaw JT: A genomewide search for type 2 diabetes-susceptibility genes in indigenous Australians. *Am J Hum Genet* 70:349–357, 2002
21. Lindgren CM, Mahtani MM, Widen E, McCarthy MI, Daly MJ, Kirby A, Reeve MP, Kruglyak L, Parker A, Meyer J, Almgren P, Lehto M, Kanninen T, Tuomi T, Groop LC, Lander ES: Genomewide search for type 2 diabetes mellitus susceptibility loci in Finnish families: the Botnia study. *Am J Hum Genet* 70:509–516, 2002
22. Meigs JB, Panhuysen CI, Myers RH, Wilson PW, Cupples LA: A genome-wide scan for loci linked to plasma levels of glucose and HbA(1c) in a community-based sample of Caucasian pedigrees: the Framingham Offspring Study. *Diabetes* 51:833–840, 2002
23. Hsueh WC, St Jean PL, Mitchell BD, Pollin TI, Knowler WC, Ehm MG, Bell CJ, Sakul H, Wagner MJ, Burns DK, Shuldiner AR: Genome-wide and fine-mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish: evidence for a new diabetes locus on chromosome 14q11 and confirmation of a locus on chromosome 1q21–q24. *Diabetes* 52:550–557, 2003
24. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI: Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26:163–175, 2000
25. Altmuller J, Palmer LJ, Fischer G, Scherb H, Wjst M: Genomewide scans of complex human diseases: true linkage is hard to find. *Am J Hum Genet* 69:936–950, 2001
26. Ji L, Malecki M, Warram JH, Yang Y, Rich SS, Krolewski AS: New susceptibility locus for NIDDM is localized to human chromosome 20q. *Diabetes* 46:876–881, 1997
27. Bowden DW, Sale M, Howard TD, Qadri A, Spray BJ, Rothschild CB, Akots G, Rich SS, Freedman BI: Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. *Diabetes* 46:882–886, 1997
28. Zouali H, Hani EH, Philippi A, Vionnet N, Beckmann JS, Demenais F, Froguel P: A susceptibility locus for early-onset non-insulin dependent (type 2) diabetes mellitus maps to chromosome 20q, proximal to the phosphoenolpyruvate carboxykinase gene. *Hum Mol Genet* 6:1401–1408, 1997
29. Ghosh S, Watanabe RM, Hauser ER, Valle T, Magnuson VL, Erdos MR, Langefeld CD, Balow J Jr, Ally DS, Kohtamaki K, Chines P, Birznieks G, Kaleta HS, Musick A, Te C, Tannenbaum J, Eldridge W, Shapiro S, Martin C, Witt A, So A, Chang J, Shurtleff B, Porter R, Boehnke M, et al: Type 2 diabetes: evidence for linkage on chromosome 20 in 716 Finnish affected sib pairs. *Proc Natl Acad Sci U S A* 96:2198–2203, 1999
30. Klupa T, Malecki MT, Pezzolesi M, Ji L, Curtis S, Langefeld CD, Rich SS, Warram JH, Krolewski AS: Further evidence for a susceptibility locus for type 2 diabetes on chromosome 20q13.1-q13.2. *Diabetes* 49:2212–2216, 2000
31. Klupa T, Warram JH, Antonellis A, Pezzolesi M, Nam M, Malecki MT, Doria A, Rich SS, Krolewski AS: Determinants of the development of diabetes (maturity-onset diabetes of the young 3) in carriers of HNF-1alpha mutations: evidence for parent-of-origin effect. *Diabetes Care* 25:2292–2301, 2002
32. Harris MI, Hadden WC, Knowler WC, Bennett PH: International criteria for the diagnosis of diabetes and impaired glucose tolerance. *Diabetes Care* 8:562–567, 1985
33. Mukhopadhyay N, Almasy L, Schroeder M, Mulvihill WP, Weeks DE: Mega2, a data-handling program for facilitating genetic linkage and association analyses. *Am J Hum Genet* 65:A436, 1999
34. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES: Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58:1347–1363, 1996
35. Kong A, Cox NJ: Allele-sharing models: LOD scores and accurate linkage tests. *Am J Hum Genet* 61:1179–1188, 1997
36. Fajans SS, Bell GI, Polonsky KS: Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 345:971–980, 2001
37. Munro S, Cuthbertson DJ, Cunningham J, Sales M, Cohen PT: Human skeletal muscle expresses a glycogen-targeting subunit of PP1 that is identical to the insulin-sensitive glycogen-targeting subunit G(L) of liver. *Diabetes* 51:591–598, 2002
38. Hisatomi M, Hidaka H, Niki I: Ca2+/calmodulin and cyclic 3, 5′ adenosine monophosphate control movement of secretory granules through protein phosphorylation/dephosphorylation in the pancreatic beta-cell. *Endocrinology* 137:4644–4649, 1996
39. Schork NJ, Boehnke M, Terwilliger JD, Ott J: Two-trait-locus analysis: a powerful strategy for mapping complex genetic traits. *Am J Hum Genet* 53:1127–1136, 1993
40. MacLean CJ, Sham PC, Kendler KS: Joint linkage of multiple loci for a complex disorder. *Am J Hum Genet* 53:353–366, 1993
41. Cox NJ, Frigge M, Nicolae DL, Concannon P, Hanis CL, Bell GI, Kong A: Loci on chromosomes 2 (NIDDM1) and 15 interact to increase susceptibility to diabetes in Mexican Americans. *Nat Genet* 21:213–215, 1999