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Localization of a Susceptibility Gene for Type 2 Diabetes to Chromosome 5q34–q35.2

Inga Reynisdottir,[1] Gudmar Thorleifsson,[1] Rafn Benediktsson,[2,3] Gunnar Sigurdsson,[2,3] Valur Emilsson,[1] Anna Sigurlin Einarsdottir,[1] Eyrun Edda Hjorleifsdottir,[1] Gudbjorg Th. Orlygsdottir,[1] Gudrun Thora Bjornsdottir,[2] Jona Saemundsdottir,[1] Skarphedinn Halldorsson,[1] Soffia Hrafnkelsdottir,[1] Steinunn Bjorg Sigurjonsdottir,[1] Svana Steinsdottir,[1] Mitchell Martin,[4] Jarema P. Kochan,[4] Brian K. Rhees,[4] Struan F. A. Grant,[1] Michael L. Frigge,[1] Augustine Kong,[1] Vilmundur Gudnason,[2] Kari Stefansson,[1,*] and Jeffrey R. Gulcher[1,*]

[1]deCODE Genetics, [2]Icelandic Heart Association, and [3]Landspitali-University Hospital, Reykjavik; and [4]Hoffmann-La Roche, Nutley, NJ

We report a genomewide linkage study of type 2 diabetes (T2D [MIM 125853]) in the Icelandic population. A list of type 2 diabetics was cross-matched with a computerized genealogical database clustering 763 type 2 diabetics into 227 families. The diabetic patients and their relatives were genotyped with 906 microsatellite markers. A nonparametric multipoint linkage analysis yielded linkage to 5q34–q35.2 (LOD = 2.90, $P = 1.29 \times 10^{-4}$) in all diabetics. Since obesity, here defined as body mass index (BMI) $\geqslant$30 kg/m^2, is a key risk factor for the development of T2D, we studied the data either independently of BMI or by stratifying the patient group as obese (BMI $\geqslant$30) or nonobese (BMI <30). A nonparametric multipoint linkage analysis yielded linkage to 5q34–q35.2 (LOD = 3.64, $P = 2.12 \times 10^{-5}$) in the nonobese diabetics. No linkage was observed in this region for the obese diabetics. Linkage analysis conditioning on maternal transmission to the nonobese diabetics resulted in a LOD score of 3.48 ($P = 3.12 \times 10^{-5}$) in the same region, whereas conditioning on paternal transmission led to a substantial drop in the LOD score. Finally, we observed potential interactions between the 5q locus and two T2D susceptibility loci, previously mapped in other populations.

Introduction

Type 2 diabetes (T2D) is a complex genetic disease, characterized by hyperglycemia due to insulin resistance in target tissues, that is usually brought to light by coexisting impairment of insulin secretion. T2D has serious consequences for health, leading to complications such as myocardial infarction, stroke, peripheral arterial occlusive disease, nephropathy, retinopathy, and neuropathy. In 1995, there were 135 million adults suffering from this disease worldwide, and the prevalence is increasing. It is estimated that close to 300 million will develop diabetes by the year 2025 (King et al. 1998). In the United States alone, it is estimated that there are 16 million diabetics, and approximately half of them remain undiagnosed (Moneva and Dagogo-Jack 2002). In fact, the average delay between onset of disease and diagnosis of T2D in the U.S. is estimated to be 10 years. Early diagnosis and treatment of T2D is important, as this allows marked reduction in complications (UKPDS33 1998), and recent studies have shown that lifestyle modifications are effective in preventing conversion of prediabetes (Knowler et al. 2002).

Both genetic and environmental factors influence disease susceptibility. Obesity is a leading risk factor, but aging, ethnicity, Western-style diet, and a sedentary lifestyle are also important. Individuals from families with a history of diabetes are at a higher risk of developing the disease (Rotter et al. 1992; Rewers and Hamman 1995; Foster 1998). The high rate of concordance between MZ twins further suggests the role of genetic components in the pathogenesis of T2D (Rotter et al. 1992; Rewers and Hamman 1995 and references therein; Medici et al. 1999; Poulsen et al. 1999).

Variants in six genes have been identified that lead to maturity-onset diabetes of the young (MODY [MIM 606391]), which is an early-onset monogenic form of type 2 diabetes. MODY 1–6 (MIM 125850, 125851, 600496, 606392, 604284, 606394) result from mutations in *HNF4α* (Yamagata et al. 1996*a*), glucokinase

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Address for correspondence and reprints: Dr. Inga Reynisdottir, deCODE Genetics, Inc., Sturlugata 8, 101 Reykjavik, Iceland. E-mail: ingar@decode.is; or Dr. Rafn Benediktsson, Icelandic Heart Association, Holtasmari 1, 201 Kopavogur, Iceland. E-mail: rafn@efnaskipti.com

* These authors contributed equally to this work.

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(Froguel et al. 1992), *HNF1α* (Yamagata et al. 1996*b*), *IPF1* (Stoffers et al. 1997), *HNF1β* (Horikawa et al. 1997), and *NEUROD1* (Malecki et al. 1999), respectively. Mutations in any of the six genes affect glucose-stimulated insulin secretion from pancreatic islet β-cells, but they do not appear to play a role in the more common forms of T2D.

A number of genes with potential roles in the pathogenesis of diabetes have been screened for mutations. Mutations have been identified in genes such as the insulin receptor (MIM 147670), but they are responsible for severe forms of diabetes with Mendelian inheritance and account for only a very small number of cases (Kahn et al. 1996). There have been numerous studies of associations of candidate genes with T2D. Most show mild or modest association with the disease. One study (Altshuler et al. 2000) reviewed some of the association work that had been done at that point and concluded that association with only 1 of 16 reported genes met the standard of replication. In a meta-analysis of previous studies, Altshuler et al. concluded that the Pro12Ala polymorphism in *PPARγ* (MIM 601487) is associated with T2D, conferring a relative risk of 1.2.

Genomewide scans have been performed in a variety of populations, revealing a number of significant or suggestive diabetes susceptibility loci. Studies in Mexican Americans have mapped genes to 2q37 (Hanis et al. 1996), 15q21 (Cox et al. 1999), 10q26 (Duggirala et al. 1999), and 3p (Ehm et al. 2000). In Pima Indians, linkages have been observed with 1q21–23 and 11q23–q25 (Hanson et al. 1998). In white populations, linkages have been established with 12q24 (Mahtani et al. 1996) and 9p13–q21 (Lindgren et al. 2002*a*) in Finns, 1q21–q23 in Americans in Utah (Elbein et al. 1999), 3q27-qter in French families (Vionnet et al. 2000), and 18p11 in Scandinavians (Parker et al. 2001). A locus was reported, in indigenous Australians, on 2q24.3 (Busfield et al. 2002). Some of the loci mentioned above have been replicated. There are also many reports of weaker linkages to other regions in the genome.

Only one of the genomewide scans has resulted in the isolation of a gene that is thought to contribute to the late-onset form of diabetes (Horikawa et al. 2000). Here, the intronic variation UCSNP-43 (G/A) in the calpain 10 gene (*CAPN10* [MIM 605286]) at the *NIDDM1* locus, was shown to be significantly associated with diabetes susceptibility among Mexican Americans. Association to UCSNP-43 has been replicated only in some follow-up studies (Baier et al. 2000; Evans et al. 2001; Tsai et al. 2001; Rasmussen et al. 2002), but variants in *CAPN10* have been associated with an increased risk of T2D in some populations (Horikawa et al. 2000; Evans et al. 2001; Malecki et al. 2001; Cassell et al. 2002; Garant et al. 2002; Lynn et al. 2002; Orho-Melander et al. 2002; Wang et al. 2002). Moreover, the at-risk allele was shown to be associated with impaired regulation of glucose-induced insulin secretion (Sreenan et al. 2001; Lynn et al. 2002) and decreased rate of insulin-stimulated glucose disposal in muscle (Baier et al. 2000; Sreenan et al. 2001).

This paper reports a genomewide search for causative genes in T2D in Iceland, stratified according to BMI. The prevalence of T2D in Iceland is relatively low, at 2.5% (Vilbergsson et al. 1997). Accordingly, ~5,000 people in Iceland aged >34 years have T2D. However, only 60% of these individuals have been diagnosed. Approximately 17% of the adult population in Iceland is obese (BMI ⩾30), and the incidence of obesity among children and adolescents is increasing dramatically (the International Obesity Task Force). Given the strong association of obesity with diabetes, we expect the incidence of T2D to increase significantly in Iceland over the next decade.

Subjects and Methods

Patients

This study was approved by the Data Protection Commission of Iceland and the National Bioethics Committee of Iceland. All patients and their relatives who participated in the study gave informed consent. All personal identifiers associated with blood samples, medical information, and genealogy were first encrypted by the Data Protection Commission, using a third-party encryption system (Gulcher et al. 2000).

This study was based on a list of 2,400 diabetics diagnosed either through a long-term epidemiology study done at the Icelandic Heart Association over the past 30 years or at one of two major hospitals in Reykjavík over the past 12 years. Two-thirds of these patients are alive, representing about half of the population of known diabetics. The majority of the patients have been contacted for this study, and the cooperation rate exceeded 90%.

All participants answered a questionnaire, including questions about medication and age at diagnosis. Their height (m) and weight (kg) were measured to calculate BMI. For known diabetics, dietary treatment with or without oral glucose-lowering agents identified T2D. Individuals currently treated with insulin were classified as having T2D if they were also using or had previously used oral glucose-lowering agents. The majority of patients on medication take oral glucose-lowering agents, and only a few patients need insulin. Although the prevalence of LADA (latent autoimmune diabetes in adults [MIM 222100, 138275]) in Iceland is unknown, this is generally <10% in Europeans (Tuomi et al. 1999). For hitherto undiagnosed individuals, the diagnosis of T2D and impaired fasting glucose (IFG) was based on the criteria set by the American Diabetes Association (Expert

Committee on the Diagnosis and Classification of Diabetes Mellitus 1997). Thus, T2D denotes all known diabetics, as well as all previously undiagnosed individuals with fasting serum glucose ⩾7 mM on two separate occasions. A diagnosis of IFG was given to individuals with fasting serum glucose between 6.1 and 6.9 mM on two occasions or >7 mM on an initial measurement and between 6.1 and 6.9 on a second measurement. The criterion for being recalled was an initial fasting serum glucose ⩾6.1 mM. Oral glucose–tolerance tests (OGTT) were not done, but known diabetics might have been previously diagnosed by an OGTT. Although the diagnosis of MODY includes onset before the age of 25 years, this is not part of the definition of T2D. In the current study, all patients had an adult onset, with only a small percentage (<3%) of T2D patients diagnosed before the age of 35 years, thus reducing the likelihood that they have MODY. We, however, can not fully exclude LADA, as referred to above.

The average age of the diabetic patients is 68.5 years (table 1). Therefore, very few patients had a parent or parents who were alive and/or were able to participate in the study. In all, 60 fathers and 98 mothers participated in the study and were genotyped. Thirteen patients had both parents affected with T2D. The diabetic status of nonparticipating parents is unknown.

Phenotypes and Pedigrees

Blood samples were collected from 964 individuals with T2D and 203 individuals with IFG. The patients with T2D were treated as probands and were clustered into families by use of our computerized genealogy database, with the criterion that each proband is related to at least one other proband by six or fewer meiotic events. The IFG patients were added to the families if they were related to a proband by three or fewer meiotic events. The rationale behind this was to include as many patients as possible in the study. Impaired fasting glucose is an intermediate diagnosis and a prediabetic state, and we reasoned that the more closely related these patients are to the diabetics, the more likely they are to develop the disease. Patients who clustered within extended families with multiple affected individuals were genotyped first; a total of 772 individuals—705 patients with T2D and 67 patients with IFG. The 259 patients with T2D who do not cluster within six meiotic events were genotyped later, since they did not contribute to the linkage analysis.

The families were checked for relationship errors by comparison of the identity-by-state (IBS) distribution for the set of 906 markers, for each pair of related and genotyped individuals, to a reference distribution corresponding to the particular degree of relatedness reflected in the genealogy database. The reference distributions were constructed from a large subset of the Icelandic population. Individuals were excluded from the study if their relationship with the rest of the family was inconsistent with the relationship specified in the genealogy database. The remaining material that was available for the study consisted of 763 patients (table 1) in 227 families, together with 764 genotyped relatives. Of the patients, 703 were confirmed patients with T2D and 60 were confirmed patients with IFG.

Table 1

T2D and IFG Patients Included in the Genomewide Analysis

	Results for Patient Group		
	All Patients	T2D	IFG
Gender (male/female)	423/340	391/312	32/28
Age (years, mean ± SD)	68.5 ± 12.2	68.9 ± 11.7	63.2 ± 16.6
BMI (kg/m^2, mean ± SD)	29.1 ± 4.8	29.2 ± 4.9	28.3 ± 4.5

Stratification of Patients Based on BMI Values

The patients were classified into two subgroups based on their BMI values: nonobese diabetics were defined as patients with BMI <30, and obese diabetics are patients who have BMI ⩾30 (obesity is defined as BMI ⩾30; see Bray [1987]). For each linkage study, the two subgroups were considered separately. Thus, we clustered by affected status (T2D) independently of BMI, and then we selected a phenotype on the basis of BMI, as described above. In other words, in a genome scan that uses nonobese diabetics, the allele sharing of obese diabetics does not contribute to the linkage. By selecting a specific subphenotype for linkage analysis, some families no longer contain a pair of related patients classified as affecteds and hence do not contribute to the linkage analysis. Table 2 summarizes the number of families and patients who contribute to each of the three patient sets used.

Genomewide Scan

A genomewide scan was performed on 772 patients and their relatives. Nine patients were excluded because of inheritance errors, so the linkage analysis was performed with 763 patients and 764 relatives. The procedure was as described in a recent linkage study in stroke (Gretarsdottir et al. 2002). In short, the DNA was genotyped with our framework marker set of 906 microsatellite markers, with an average resolution of 4 cM. Alleles were called automatically with the TrueAllele program (Cybergenetics), and the program DecodeGT was used to fractionate according to quality and to edit the called genotypes (Palsson et al. 1999). The population allele frequencies of the markers were constructed from a cohort of >30,000 Icelanders who had participated in genomewide studies of various disease projects at deCODE Genetics. Additional markers were geno-

Table 2

Numbers of Patients, Pairs of Patients, and Families with T2D and IFG Contributing to the Linkage Analysis

Phenotypes	Total No. of Patients	No. of Families Contributing to the Analysis	No. of Patients Contributing to the Analysis	No. of Pairs Contributing to the Analysis
All diabetics	763	227	763	1,213
Obese diabetics	296	92	219	215
Nonobese diabetics	467	154	413	482

NOTE.—The number of families included in the analysis of the obese and nonobese subphenotypes does not add up to the number of families when the material is analyzed as a whole, as some families may include pairs of related obese patients and pairs of related nonobese patients. Likewise, not all patients are related to another patient of the same subphenotype, and such patients are excluded from the analysis of the subphenotypes.

typed within the loci on chromosomes 5q, 10q, and 12q to increase the information on identity by descent within the families. For those markers, at least 180 Icelandic controls were genotyped to derive the population allele frequencies.

The additional microsatellite markers that were genotyped within a locus were either publicly available or designed by us (in-house markers with a DG designation; see deCODE Genetics Web site). Repeats within the DNA sequence were identified that allowed us to choose or design primers that are evenly spaced across the locus. The identification of the repeats and location with respect to other markers was based on the work of the physical mapping team at deCODE. The team has reassembled a physical map on the basis of the finger printed contigs (FPC) from Santa Cruz and the published sequence of the human genome (UCSC Genome Bioinformatics).

For the markers in the framework map, the genetic locations were taken from the recently published high-resolution genetic map (HRGM) constructed at deCODE Genetics (Kong et al. 2002). However, only some of the markers added to the loci on 5q, 10q, and 12q are included in the HRGM. Markers that are not present in the HRGM are placed according to their physical location in the published sequence of the human genome. The corresponding marker order was verified, and the intermarker distances were estimated by use of genetic mapping, on the basis of the familial data collected for the T2D study. The same methods were used for the genetic mapping as were used in constructing the HRGM (Kong et al. 2002). The order of the markers within the locus on chromosome 5 and the genetic distances between them can be found on the deCODE Genetics Web site. The genetic distances have been converted to Kosambi units for the publication, in accordance with the published deCODE map; however, the actual linkage calculations were done by use of a Haldane map function, under the assumption of a no-interference model.

Statistical Methods for Linkage Analysis

Multipoint, affected-only allele-sharing methods were used in the analyses to assess evidence for linkage. All results, both the LOD score and the nonparametric linkage (NPL) score, were obtained by use of the program Allegro (Gudbjartsson et al. 2000). Our baseline linkage analysis, as described elsewhere (Gretarsdottir et al. 2002), uses the S_{pairs} scoring function (Whittemore and Halpern 1994; Kruglyak et al. 1996), the exponential allele-sharing model (Kong and Cox 1997), and a family weighting scheme that is halfway, on the log-scale, between weighting each affected pair equally and weighting each family equally. The information measure we use is part of the Allegro program output, and the information value equals zero if the marker genotypes are completely uninformative and equals one if the genotypes determine the exact amount of allele sharing by descent among the affected relatives (Gretarsdottir et al. 2002). We computed the *P* values two different ways and here report the less significant result. The first *P* value was computed on the basis of large sample theory; the distribution of $Z_{lr} = \sqrt{2[\log_e(10)\text{LOD}]}$ approximates a standard normal variable under the null hypothesis of no linkage (Kong and Cox 1997). The second *P* value was calculated by comparison of the observed LOD score with its complete data-sampling distribution under the null hypothesis (Gudbjartsson et al. 2000). When the data consist of more than a few families, as is the case here, these two *P* values tend to be very similar.

The possibility of imprinting effects on the observed loci was investigated. Implemented in our linkage analysis program, Allegro, are imprinting-based scoring functions that allow us to assign weights to allele sharing specific to parental origin. For example, to investigate paternal imprinting, the scoring function only considers the sharing of alleles transmitted to two affected relatives through their fathers. When these imprinting-based scoring functions were used, sex-specific genetic maps were

used in the calculations (Kong et al. 2002; Karason et al. 2003).

Interaction between Loci

To assess the relationship between pairs of loci, and to identify possible interaction between loci, an analysis was carried out conditional on the evidence for linkage at the locus on 5q. We then observed what effect this had on other loci identified in the genomewide linkage analysis—that is, on 6q, 10q, and 12q. The positive interactions (e.g., epistasis) were modeled by assigning weight 0 to families with an NPL score ≤ 0. Negative interactions or heterogeneity were modeled by assigning weight 0 to families with a linkage score ≥ 0 (Cox et al. 1999). The family NPL score was used for calculating the correlation, as this has a more appropriate distribution (mean 0, variance 1, with no missing information) than the LOD score. Furthermore, the family NPL scores are independent of each other, unlike the family LOD scores. Where an increase in the LOD score was observed, the significance is determined by simulations. A subset of families is selected that corresponds to the number of families with positive (or negative) NPL score, and the linkage analysis is repeated using only those families. This procedure was repeated 1,000 times and the *P* value presented is the fraction of the 1,000 simulations that produced, at the peak locus, a LOD score greater or equal to that observed when the analysis is restricted to the families with a positive (or negative) NPL score.

Results

The genealogy database was used to cluster the patients confirmed as having T2D into families, in which each patient was related to at least one other patient by six or fewer meiotic events. The patients with IFG (see the "Methods" section) were added to the families if they were related to a patient with T2D by three or fewer meiotic events. The genome scan was performed with 763 patients who clustered into 227 families. The mean separation of affected pairs was 4.9 meiotic events. The analysis was performed with three phenotypes: all patients with T2D, nonobese diabetics, and obese diabetics. In the nonobese category were 60% of our patients: 20% with BMI <25 (lean) and 40% with BMI ≥ 25 but <30 (overweight), and in the obese category were 40% of our patients: 40% have BMI ≥ 30. The number of patients and families in each category is shown in table 2. Figure 1 depicts the allele-sharing LOD scores from the analysis with the framework marker set. It shows that the strongest linkage for all T2D is to chromosome 5q. The best LOD scores using the obese diabetics were 1.14 and 1.30 on chromosome 11p (D11S4149 and D11S928, respectively), 1.05 on chromosome 12q (D12S346) and 1.31 on chromosome 18p (D18S63). The results from the analysis with nonobese diabetics are reported below.

Chromosome 5

A LOD score of 1.84 is observed on chromosome 5q34-q35.2 with the framework marker set when all the diabetic patients are included in the analysis (fig. 1). When the linkage analysis is restricted to nonobese diabetics, this LOD score increases to 2.81, still with the framework marker set. The obese diabetics do not show linkage to this region.

The information on identity by descent in the region on 5q34–q35.2 was only ~78%, with the framework marker set. Thus, an additional 38 microsatellite markers in a 40-cM region around the peak were genotyped, which resulted in an increase in the information content to ~95%. With the additional markers, the peak LOD score increased to 3.64 ($P = 2.12 \times 10^{-5}$) for the nonobese diabetics (fig. 2). For all of the patients, the peak LOD score increased to 2.90 ($P = 1.29 \times 10^{-4}$). The peak is centered on marker D5S625, and a drop of 1 in the LOD score is the region determined by the boundary markers DG5S5 and D5S429, centromeric and telomeric, respectively. This region is ~9 cM in size, and we estimate it to be ~3.7 Mb. Markers with the prefix DG5S are markers that we designed for repeat elements within the locus and can be found on the deCODE Genetics Web site (see the "Subjects and Methods" section).

Interactions between Loci

The most prominent additional LOD scores observed using the nonobese diabetic phenotype were a LOD score of 1.80 on 6q (marker D6S1569), a LOD score of 1.69 on 10q (marker D10S1773) and a LOD score of 1.44 on 12q (marker D12S366). The interactions between these three loci and the locus on chromosome 5q were studied. The families were stratified on the basis of their family NPL score at the peak marker (D5S625) on 5q, into families with positive NPL score (63 families and 191 nonobese diabetics) and families with negative NPL score (88 families and 246 nonobese diabetics). The linkage analysis was repeated with those two subgroups of families separately and LOD score changes at these three loci were investigated. For two of the loci, an increase in the LOD score was observed. A negative interaction was observed for the locus on chromosome 10q; linkage analysis of the negative-NPL families increased the LOD score to 4.06 at marker D10S1693. For the locus on chromosome 12q a positive interaction was observed; linkage analysis of the positive-NPL families increased the LOD score to 1.91 at marker

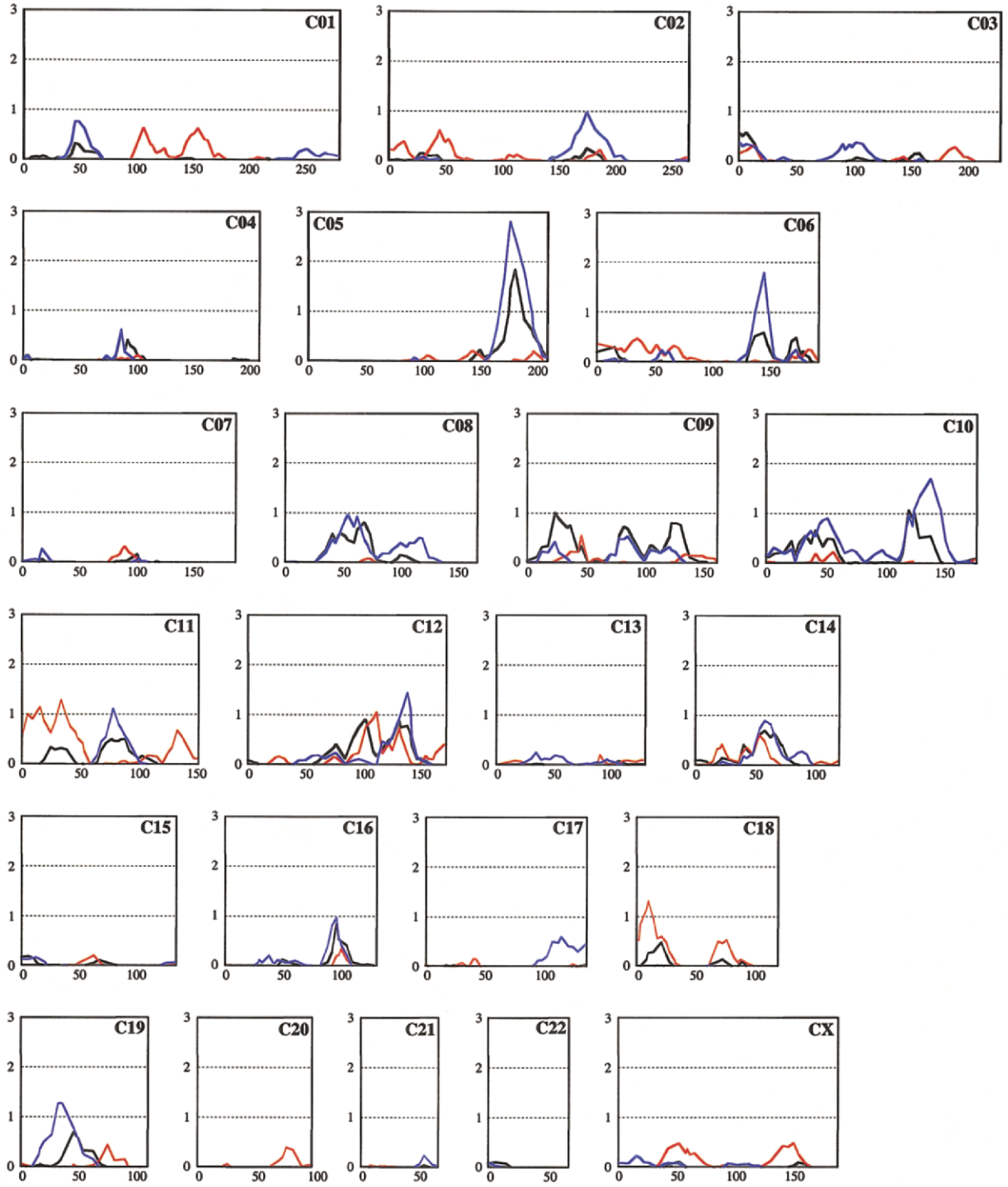

Figure 1 Genomewide scan using 906 microsatellite markers. Results are shown for three phenotypes: all type 2 diabetics (*black lines*), obese diabetics (*red lines*), and nonobese diabetics (*blue lines*). The multipoint LOD score is on the vertical axis, and the centiMorgan distance from the p-terminus of the chromosome is on the horizontal axis.

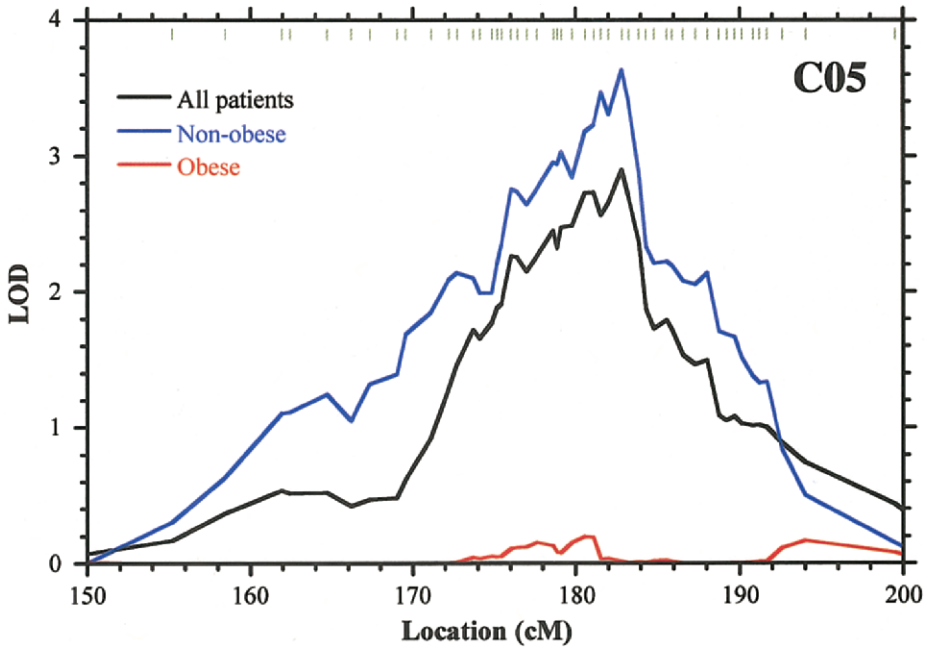

Figure 2 Multipoint allele-sharing LOD score of a portion of chromosome 5 after 38 microsatellite markers have been added to the framework marker set in a 40-cM interval from 160 cM to 200 cM. The average distance between the markers in the peak reagion is 0.84 cM. Results are shown for the same three phenotypes as in figure 1, and the location of the markers is indicated with green vertical bars at the top of the figure.

D12S366. The LOD score at the chromosome 6q locus decreased in both cases.

Additional markers were genotyped within the loci on chromosomes 10 and 12. Twenty-one additional markers were genotyped in a 20-cM region around the peak marker D12S366 on 12q. This raised the information content in the linkage analysis to ~97%. Without restricting the analysis to families with a positive NPL score on chromosome 5q, the LOD score was 1.47, including the additional markers now centered at marker DG12S125 (fig. 3*a*). However, when the positive-NPL families were analyzed, the LOD score in the region increased to 2.26, also at DG12S125. The significance of this increase was examined by 1,000 simulations, selecting 63 families at random each time and repeating the linkage analysis. Only 20 of 1,000 simulations ($P = .02$) resulted in a LOD score that was equal or higher than the observed LOD score of 2.26.

An additional 8 markers were genotyped in a 20-cM region around the peak on 10q. This increased the information content in the linkage analysis to ~86% around the peak region of the locus. The LOD score was 1.24 when all families with nonobese diabetics were used (fig. 3*b*). Analysis restricted to the negative-NPL families increased the maximum LOD score to 2.79 (still at marker D10S1693), although this increase is smaller than what we observed when using only the framework marker set. The significance of this LOD score was tested by performing 1,000 simulations. Here, 74 of the 1,000 simulations yielded a maximum LOD score of 2.79 or higher (i.e., a P value of .074).

Imprinting

The locus on chromosome 5q was affected dramatically when imprinting was considered. The linkage analysis using only maternal transmission to nonobese diabetics gave a LOD score of 3.48 (fig. 4) or $P = 3.12 \times 10^{-5}$, at marker DG5S45, a shift in location of 2 cM from the peak obtained using all the nonobese diabetics (see marker map at the deCODE Genetics Web site). In contrast, when only the paternal transmissions were considered, the maximum LOD score was 0.53 at marker DG5S910. Similar numbers of affected pairs are related through the maternal lineage (194 pairs) and the paternal lineage (190 pairs).

Furthermore, an effect of imprinting was observed on the loci on chromosome 12 in the nonobese diabetics and chromosome 18 in the obese diabetics (data not shown). When paternal transmission to nonobese diabetics was considered, the LOD score on chromosome 12q was 2.41, with the framework marker set at marker D12S79, a shift of 5 cM from the peak marker. Inclusion of the additional 21 microsatellite markers in the anal-

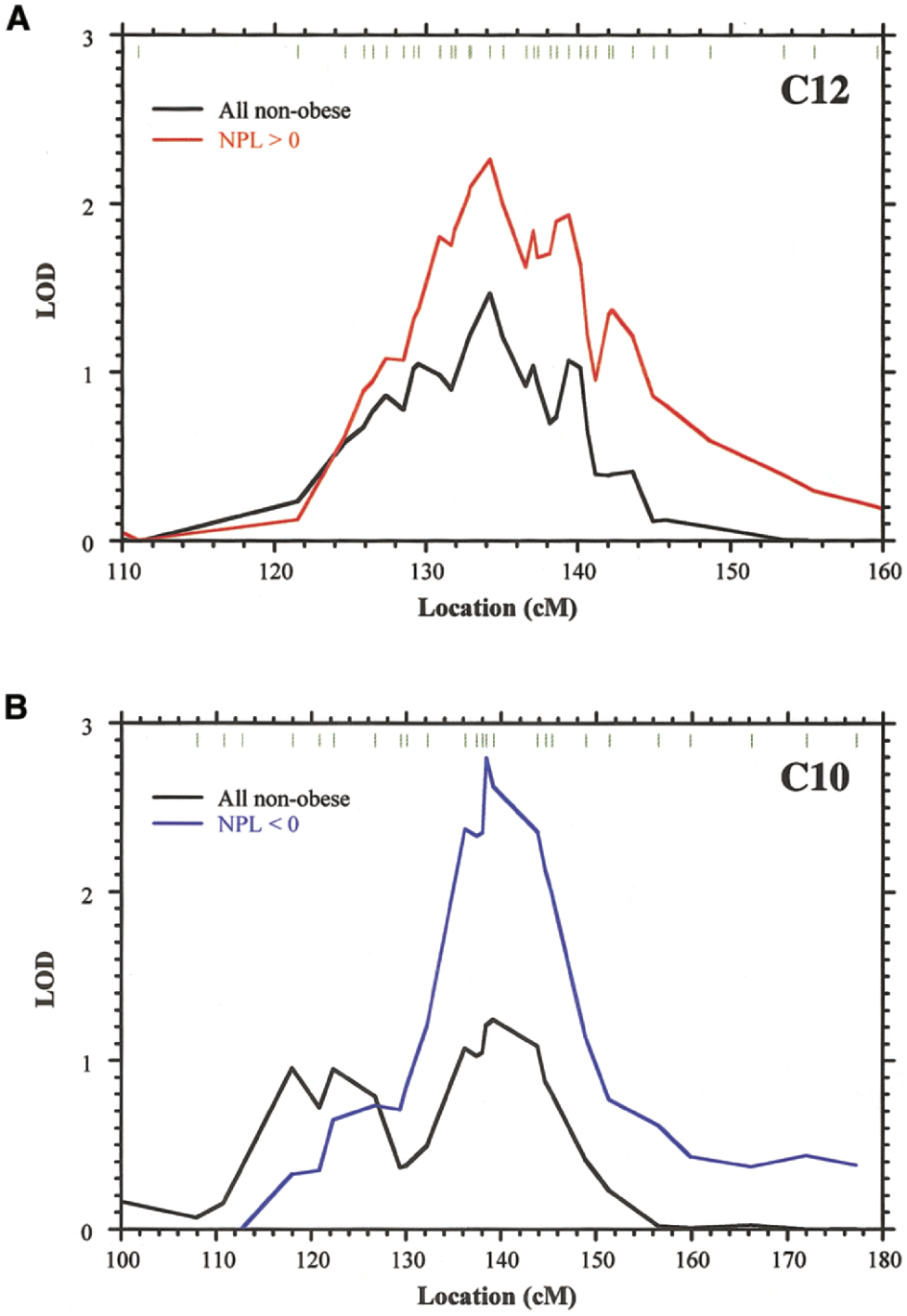

Figure 3 *a*, Positive interaction between the loci on chromosomes 5 and 12. Multipoint allele-sharing LOD score of a portion of chromosome 12 after 21 microsatellite markers have been added to the framework marker set. Results are shown for all nonobese diabetics (*black line*) and for nonobese diabetics in positive-NPL families on chromosome 5 (*red line*). *b*, Negative interaction between the loci on chromosomes 5 and 10. Multipoint allele-sharing LOD score of a portion of chromosome 10 after 8 microsatellite markers have been added to the framework marker set. Results are shown for all nonobese diabetics (*black line*) and for nonobese diabetics in negative-NPL families on chromosome 5 (*blue line*).

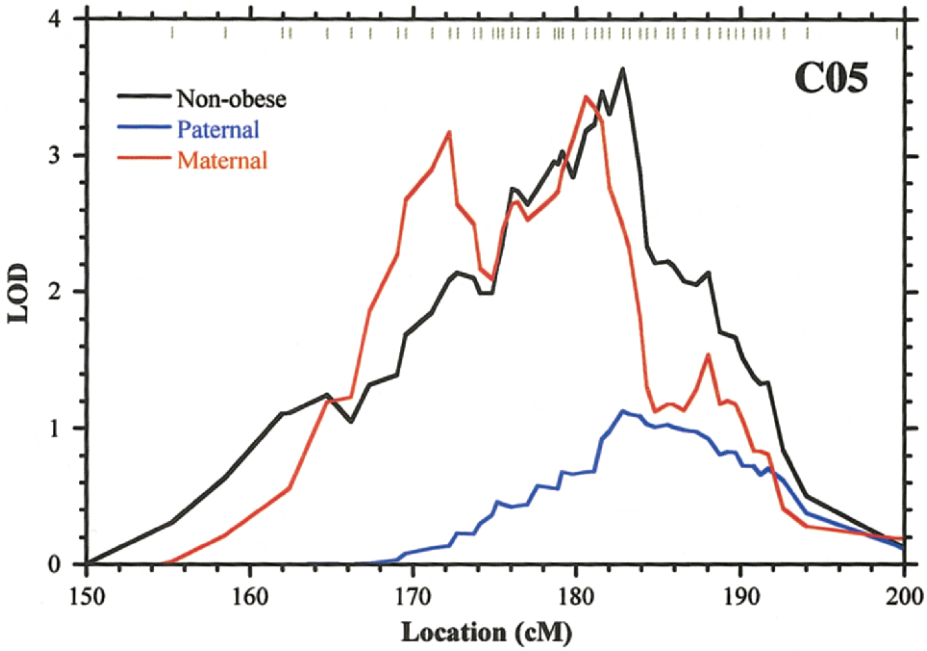

Figure 4 The effects of imprinting on the LOD score on chromosome 5. Maternal versus paternal effect on chromosome 5 locus. Results are shown for all nonobese diabetics (*black line*), nonobese diabetics with maternal inheritance (*red line*), and nonobese diabetics with paternal inheritance (*blue line*).

ysis resulted in a decrease in the LOD score to 1.43 and a shift of the peak to D12S157, ~2 cM from the peak marker. However, analysis of the maternal transmission resulted in no linkage to this chromosomal region. An interesting observation came from the effect of imprinting on the linkage analysis on chromosome 18p: maternal transmission to obese diabetics resulted in an increase in the LOD score to 2.48 at marker D18S1132, which is a shift of 4.5 cM from the peak marker obtained in all obese diabetics. No linkage was observed when the paternal transmission was considered.

Discussion

A number of genomewide scans for T2D have been performed using different ethnic groups yielding several significant and suggestive T2D susceptibility loci. The genomewide scan using nonobese type 2 diabetics in Iceland resulted in a linkage to chromosome 5q34–q35.2 with a LOD score of 3.64 ($P = 2.12 \times 10^{-5}$). The corresponding LOD score for the obese type 2 diabetics was 0.2 ($P = .17$) and for the whole patient cohort the LOD score was 2.90 ($P = 1.29 \times 10^{-4}$). Note however that these LOD scores, and the corresponding *P* values, have not been corrected for the number of phenotypes analyzed and the significance should be interpreted accordingly.

Obesity, which is controlled by both genetic and environmental factors, is strongly associated with diabetes. In fact, 40% of the diabetics in Iceland are obese, which is more than twice the population prevalence of obesity (17%). Thus, we reasoned that by fractionating the patient group into nonobese and obese study groups, we might (1) decrease the effects of genetic and environmental factors that contribute directly to obesity but only indirectly to T2D and (2) perhaps select for individuals with primarily beta cell dysfunction, as they did not need as severe a level of obesity to unmask their diabetes. Suggestive evidence for linkage (LOD score >2.2) was not observed when the obese diabetic study group was used. However, the strongest linkage for that group was to chromosome 18, with a LOD score of 1.31 at marker D18S63, corresponding approximately to a previously reported linkage for obese type 2 diabetics in Scandinavia (Parker et al. 2001).

Previously published T2D genomewide scans have not demonstrated a suggestive linkage to 5q35. There are, however, few reports that have documented nominal linkage to regions that are either just centromeric or telomeric to the locus. Elbein et al. (1999), Vionnet et al. (2000), and Lindgren et al. (2002*a*) all noted a weak linkage centromeric to our peak on chromosome 5, using as affected subjects individuals with T2D or with T2D combined with glucose intolerance. In a report on early-onset diabetes (Lindgren et al. 2002*b*), the authors observe a weak linkage to an extensive region

at the q arm of chromosome 5, a region that includes our linkage peak. The subjects studied in these reports are, like the Icelandic patients, white, although coming from Utah (Elbein et al. 1999), France (Vionnet et al. 2000), Sweden, and Finland (Lindgren et al. 2002*a*; Lindgren et al. 2002*b*). Further, Luo et al. (2001) have shown some evidence for linkage to 5q in Chinese Hans with T2D. Here, the linkage is telomeric to the peak in the Icelandic material. Also, a QTL analysis in Finns (T2D and BMI) reports a weak linkage telomeric to the Icelandic linkage peak but close to the linkage peak in the Chinese Hans (Watanabe et al. 2000). The reported linkage that best overlaps with the locus reported here was observed in Pima Indians, in a study that used prediabetic phenotypes (Pratley et al. 1998). Although weak, the phenotype AIR (acute insulin response) showed linkage to marker D5S820 (LOD score = 0.70), which is at the border of the 1.5-LOD drop within our locus. It is close to marker D5S2049 in our map (see deCODE Genetics Web site). AIR is a measurement of insulin secretion, and a low AIR is a predictor of T2D in insulin-resistant Pima Indians (Thompson et al. 1995). In none of the studies was the patient material fractionated according to our criteria, with the exception of the study by Watanabe et al. (2000), who stratified the diabetics according to BMI in their QTL analysis. The only report describing linkage to this region on chromosome 5 that completely overlaps with the Icelandic locus is in a recently published paper (Frayling et al. 2003) that describes a genomewide scan in families of European descent with MODY (the multipoint NPL *Z* score was 2.04; D5S400–D5S498). The use of different populations as well as different marker orders could explain why the peaks are spread over a considerable area. It is also possible that there is more than one gene on the telomeric arm of chromosome 5q that contributes to diabetes.

It has been suggested that the effects of imprinting on the genetics of a complex disease such as diabetes have been underestimated. However, some fragmentary data do exist, showing significant effects of imprinting on the development of T2D. Thus, T2D was reported to be associated with paternally derived alleles in the VNTR of the insulin promoter (Huxtable et al. 2000). Transient neonatal diabetes mellitus (TNDM) is associated with a duplication of a segment of chromosome 6 when the duplication is of paternal origin (Gardner et al. 1999). Low birth weight predisposes to development of T2D (Barker et al. 1993). Some of the genes that are known to affect fetal growth, including *IGF2,* are predominantly paternally expressed (Huxtable et al. 2000). Our analysis of the nonobese diabetes locus on chromosome 5 suggests that imprinting could play a role. The LOD score was 3.48 when maternal transmission was considered but was only 0.53 when the paternal transmission was considered. The observation for chromosome 12 was reversed, in that a higher LOD score was obtained when a paternal transmission was considered than when a maternal transmission was considered. In the obese diabetics, a maternal transmission was observed for the locus on chromosome 18p. One genomewide analysis in Pima Indians explored the effects of imprinting on the inheritance of diabetes and BMI (Lindsay et al. 2001). None of their loci met criteria for genomewide significance, but, using maternally derived alleles, they observed linkage to age-adjusted diabetes on chromosome 5, near our locus.

Mahtani et al. (1996) reported evidence for linkage to 12q24. The *HNF1α* (*TCF1*) gene resides within this locus and mutations in this gene have been shown to cause MODY3, a rare early-onset form of T2D. The gene has been screened for association to late-onset T2D; however, results have been negative (Shaw et al. 1998). Linkage to this region was detected in our diabetes material. First, using framework markers, we observed a positive LOD score of 1.44 at 12q24, using the nonobese diabetics. Second, the 12q24 locus interacts positively with our major locus on chromosome 5, and the linkage gained increased support by the addition of more markers. When linkage analysis was performed using the nonobese diabetes families with a positive NPL scores on chromosome 5, the LOD score on 12q increased from 1.47 to 2.26. These results suggest a synergy between diabetes-susceptibility genes at 5q and 12q24.

The first and only polygene isolated for T2D through positional cloning is the calpain 10 gene at 2q37 (Horikawa et al. 2000). The original linkage was observed in Mexican Americans, but it has not been replicated in white populations (Ciccarese et al. 1997; Hani et al. 1997; Thomas et al. 1997; Ghosh et al. 1999; Ehm et al. 2000; Vionnet et al. 2000; Lindgren et al. 2002*a*), including Icelanders (i.e., in the present study). Furthermore, we have not been able to replicate the linkages reported by others to chromosomes 20, 1q, 11q, 3q, 3p, or 15q. In the present genomewide scan, linkages were observed to chromosomes 6q, 11q, 16q, and 19p (LOD scores range from 1.0 to 1.8) in the nonobese diabetics. There was a decrease in the linkage to 6q when the locus on chromosome 5 was conditioned on. Although some of these linkages could well be false-positive results, some of these weak linkages overlap with published results. The locus on 6q overlaps with the one reported by Hanson et al. (1998) in diabetic Pima siblings. They observed a linkage with a *Z* score of 1.39. The locus on 11q in nonobese diabetics overlaps somewhat with the Ghosh et al. (2000) locus on chr11 (1.75, additive model). The loci on chromosomes 16q and 19p have not been reported by others. One of the two loci observed in the obese diabetics overlaps with

a previously published locus on 18p in Scandinavian obese diabetics (Parker et al. 2001). The other locus on chromosome 11p does not overlap with a previously known locus.

Our results suggest that we have uncovered a locus on chromosome 5q that contains a gene that contributes to the development of T2D in nonobese Icelanders. This gene appears to be imprinted and to interact with a second locus on chromosome 12q.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Cybergenetics, http://www.cybgen.com/ (for True Allele program)
deCODE Genetics, http://www.decode.com/diabetes/markermaps/ (for marker maps in linkage analysis)
International Obesity Task Force, http://www.iotf.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
UCSC Genome Bioinformatics, http://genome.ucsc.edu/

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