Evidence for a Novel Type 1 Diabetes Susceptibility Locus on Chromosome 8

Michèle M. Sale,[1] Liesel M. FitzGerald,[1] Jacinta C. Charlesworth,[1] Donald W. Bowden,[2] and Stephen S. Rich[2]

Type 1 diabetes results from a combination of genetic susceptibility and environmental exposures. Susceptibility loci other than HLA and the insulin gene remain to be identified to account for the degree of familial clustering observed in this disorder. Early genome-wide scans provided suggestive evidence of linkage on chromosome 8q, prompting detailed analysis of this region. A total of 20 microsatellite markers spanning an 88-cM region of 8q11-24 were genotyped in 24 type 1 diabetes pedigrees from Wisconsin that contained 39 affected sib-pairs. Multipoint linkage analyses provided close to suggestive evidence of linkage, with a multipoint logarithm of odds score (MLS) of 2.4 and Genehunter nonparametric logarithm of odds score (NPL) of 2.7 (P = 0.003). There is also evidence of linkage disequilibrium at peak marker D8S1823 for the 217bp allele (P = 0.037) using the pedigree disequilibrium test. Although our sample size was small, the multiple tests were consistent and our preliminary results suggested that 8q24 may harbor a novel population-specific type 1 diabetes susceptibility gene. Continued investigation of this region for a novel type 1 diabetes susceptibility gene appears justified. *Diabetes* 51 (Suppl. 3): S316–S319, 2002

It is recognized that β-cell destruction observed in type 1 diabetes is the result of a combination of genetic and environmental factors. Identification of genes responsible for increased susceptibility may aid prevention or early intervention and may help elucidate the primary causes of type 1 diabetes. The genetic contribution to type 1 diabetes has been extensively studied, with the HLA region (1) and insulin gene (2) as the first identified susceptibility loci. Using genome-wide screens, candidate gene approaches, and nonobese diabetic (NOD) mouse synteny, several other type 1 diabetes loci have been reported, although few have been subsequently confirmed. It is clear that other susceptibility loci remain to be identified to account for the degree of familial clustering observed in type 1 diabetes.

Previous evidence for linkage to chromosome 8q in type 1 diabetes has been reported. Davies et al. (1) obtained a multipoint logarithm of odds score (MLS) of 2.6 in their genome-wide screen of 96 British families and also found support for linkage in their HLA-identical sib-pairs (MLS 2.0). In a follow-up study of an additional 500 affected sib-pairs (ASPs) (U.K. and U.S. combined), Cucca et al. (3) obtained MLS values on 8q of 1.2 and 1.6. In a second-generation genome-wide scan of 679 U.S. and U.K. ASPs, Concannon et al. (4) detected a logarithm of odds (LOD) score of 1.08 on 8q. These reported linkage results provided consistent evidence for the involvement of a type 1 diabetes locus with a modest effect on chromosome 8q, leading to this detailed analysis of this region.

RESEARCH DESIGN AND METHODS

Families residing in Wisconsin containing at least two siblings with type 1 diabetes (one individual taking insulin by age 20 years, with at least one other sibling taking insulin by age 30 years) were recruited, as described previously (5,6). A total of 24 pedigrees containing 39 ASPs were available for analysis. Twenty microsatellite markers across 8q11-24 were genotyped: D8S285, D8S260, D8S286, D8S525, D8S1697, D8S167, D8S273, D8S88, D8S2320, D8S1694, D8S522, D8S199, D8S1823, D8S269, D8S586, D8S1728, D8S1802, D8S514, D8S284, and D8S272. Primer sequences and PCR conditions were as described in the Genome Database (http://gdbwww.gdb.org). One primer for each microsatellite was fluorescently labeled with either FAM or HEX (Sigma, St. Louis, MO), and PCR products were genotyped using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Multipoint nonparametric linkage analysis was carried out using Genehunter 2.1 (7) estimate and nonparametric LOD (NPL) commands, using allele frequencies derived from founder individuals in the Wisconsin sample. Map order was taken from the University of California, Santa Cruz (UCSC) genome browser (http://genome.cse.ucsc.edu) and was in agreement with the Ensembl Human Genome Server sequence (http://www.ensembl.org/Homo_sapiens) except for markers D8S1823 and D8S269, which were reversed. Map distances were taken from the Marshfield chromosome 8 sex-average linkage map, except where genetic distances between adjacent markers were ≤1.15 cM (which included all markers from D8S1694 to D8S1728 across the peak), where physical distances from the UCSC sequence were used. Analysis of linkage disequilibrium (LD) was carried out using the pedigree disequilibrium test (PDT) version 2.11 (8).

RESULTS AND DISCUSSION

Multipoint linkage analyses of 39 ASPs from 24 Wisconsin pedigrees provided close to suggestive evidence of linkage, with an MLS of 2.4 (Fig. 1) and Genehunter NPL of 2.7 (P = 0.003) (Fig. 2). Reversing the order of markers D8S1823 and D8S269 did not change these values (data not shown). There is also evidence of LD at marker D8S1823 (the location of the linkage peak) for the 217-bp allele (P = 0.037) using the PDT (Table 1). This allele was present in the founder individuals (parents) from the Wisconsin

From the [1]Menzies Centre for Population Health Research, University of Tasmania, Hobart, Australia; and the [2]Wake Forest University School of Medicine, Winston-Salem, North Carolina.

Address correspondence and reprint requests to Dr. Michèle Sale, Center for Human Genomics, Wake Forest University School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157. E-mail: msale@wfubmc.edu.

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ASP, affected sib-pair; LD, linkage disequilibrium; LOD, logarithm of odds; MLS, multipoint logarithm of odds score; NPL, nonparametric logarithm of odds; PDT, pedigree disequilibrium test.

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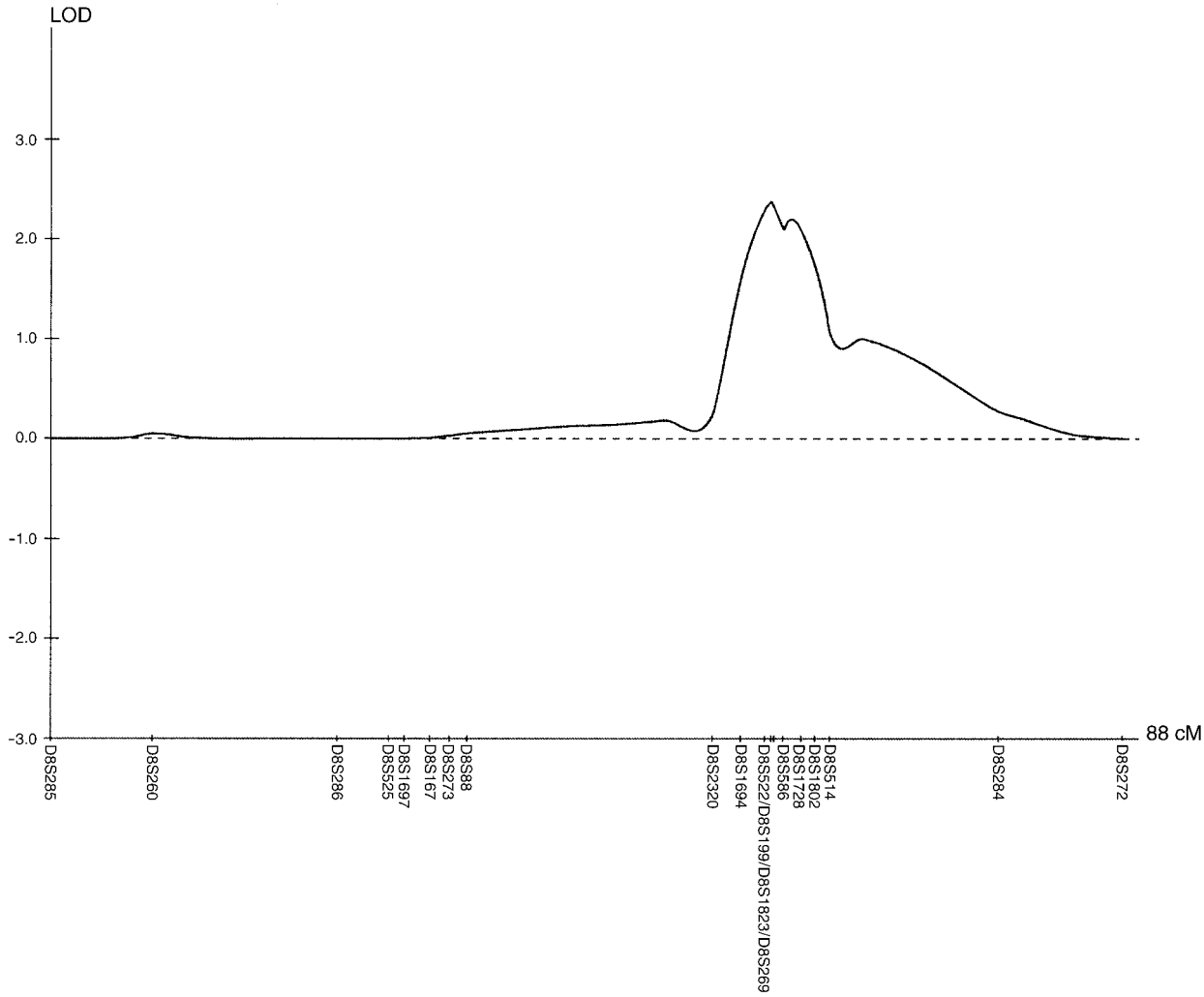

FIG. 1. MLS results obtained using the "estimate" command of Genehunter 2.1, assuming dominance variance may exist, and using allele frequencies from founder individuals (parents) in the Wisconsin dataset.

population at a frequency of 0.43. There was no evidence of LD with any of the remaining microsatellite markers. Our preliminary data therefore suggest that 8q24 may harbor a novel population-specific type 1 diabetes susceptibility gene.

The linkage peak of Davies et al. (1) at D8S556, located between our typed markers D8S88 and D8S2320, is 9 cM proximal to our peak marker D8S1823. The peak of Concannon et al. (4) at D8S1779 (genetically mapped between D8S2320 and D8S1694) is 2.5 cM proximal to D8S1823. The more distal peak of the two peaks detected by Cucca et al. (3), located between D8S198 (1.7 cM proximal to D8S1823) and D8S281 (1.2 cM distal to D8S1823), lies within our region of linkage. Additional conditional multipoint analyses of the U.K. data from Professor John Todd's group (http://www-gene.cimr.cam.ac.uk/todd) indicate that their evidence for linkage across a broad 50-cM region from D8S273 to D8S198, which encompasses the linkage peak observed in this analysis, is greatest in HLA-identical sibs (MLS 2.2) and those sharing a DR3/DR4 genotype (MLS 1.5). It is interesting to note that on stratification of this admittedly small dataset, 23 (59%) of the 39 Wisconsin ASPs shared an HLA-A/-B/-DR haplotype, and 21 ASPs (54%) shared the DR3/DR4 combination. The latter is considerably higher than the proportion of DR3/DR4 heterozygotes in a population-based sample of type 1 diabetes case subjects (38%) and control subjects (5%) from Wisconsin (9), suggesting that the putative locus on 8q24 may interact with the HLA region.

The sample size used for our study is small, especially when compared with other recent type 1 diabetes studies. The estimated locus-specific λ_S for D8S1823 (the marker closest to the linkage peak) is 1.6 and, based on the work of Risch (10–12), this result suggests that we would require ~300–400 ASPs for 95% power to detect a LOD score of 3.

The families studied were all recruited from Wisconsin. In 1989 (when the families in this investigation were recruited), the Wisconsin population could be described as fairly homogeneous, primarily middle-class, small-town or rural, and of northern European Caucasian descent (9). Additionally, in 1970–1979, southern Wisconsin had one of the highest rates of type 1 diabetes in the U.S., with an incidence of 18.2 cases (under 15 years of age) per 100,000 individuals (13). The patients and relatives studied are thus likely to possess overall reduced genetic heterogeneity, especially when compared with large published heterogeneous studies using resources such as the Human

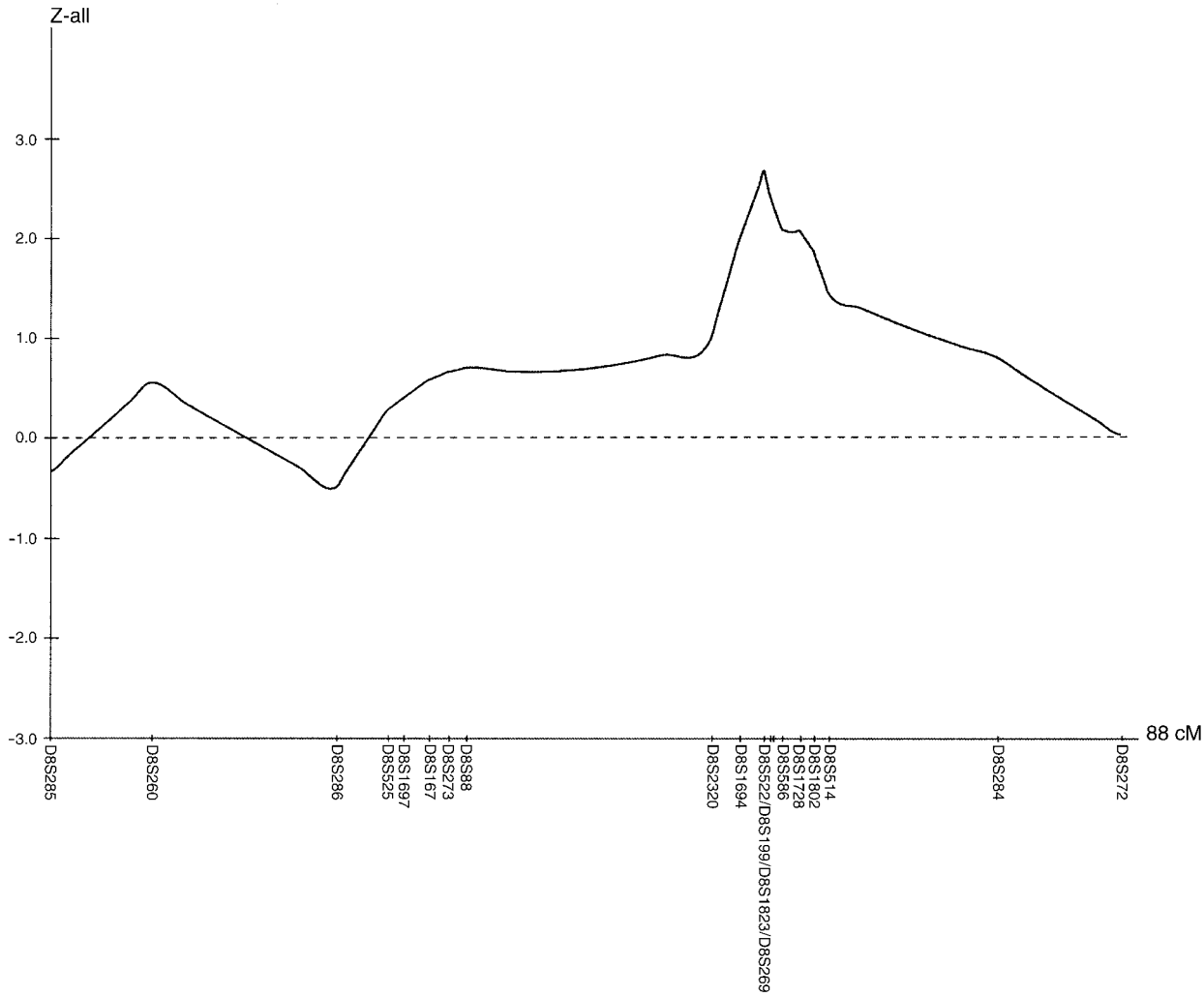

FIG. 2. Genehunter 2.1 NPL results, using founder allele frequencies.

Biological Data Interchange and U.K. Warren Repository, possibly explaining the relatively modest linkage scores achieved by other studies of this region (1,3,4). It is conceivable that expanding the original 96 Caucasian U.K.-born families (1) to 357 U.K. and 236 U.S. families (3) or 250 U.K. and 366 U.S. families (4) may have increased heterogeneity sufficiently to diminish the previously suggestive multipoint linkage value.

In the future, we intend to investigate the association in already-collected Wisconsin-born case and control subjects (14). Evaluation of other Midwest U.S. populations for comparison with this region of linkage may also be fruitful. Independent confirmation in a second dataset from a comparable population would provide considerable support for this locus. Because our results are close to suggestive linkage using the strict criteria of Lander and Kruglyak (15) and multiple approaches provide consistent evidence for a susceptibility locus, continued investigation of this region for a novel type 1 diabetes susceptibility gene is warranted.

TABLE 1
PDT results for microsatellite marker D8S1823

	df	*Z*	*P*
Allele (bp)			
217	1	2.089	0.0367
219	1	0.000	1.000
223	1	−1.136	0.2560
225	1	−1.000	0.3173
229	1	−1.633	0.1025
Global score	4	7.458	0.1136

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