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

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Despite recent advances in the molecular genetics of type 2 diabetes, the majority of susceptibility genes in humans remain to be identified. We therefore conducted a 10-cM genomewide search (401 microsatellite markers) for type 2 diabetes–related traits in 637 members of 143 French pedigrees ascertained through multiple diabetic siblings, to map such genes in the white population. Nonparametric two-point and multipoint linkage analyzes—using the MAPMAKER-SIBS (MLS) and MAXIMUM-BINOMIAL-LIKELIHOOD (MLB) programs for autosomal markers and the ASPEX program for chromosome X markers—were performed with six diabetic phenotypes: diabetes and diabetes or glucose intolerance (GI), as well as with each of the two phenotypes associated with normal body weight (body-mass index<27 kg/m^2) or early age at diagnosis (<45 years). In a second step, high-resolution genetic mapping (~2 cM) was performed in regions on chromosomes 1 and 3 loci showing the strongest linkage to diabetic traits. We found evidence for linkage with diabetes or GI diagnosed at age <45 years in 92 affected sib pairs from 55 families at the *D3S1580* locus on chromosome 3q27-qter using MAPMAKER-SIBS (MLS = 4.67, *P* = .000004), supported by the MLB statistic (MLB-LOD = 3.43, *P* = .00003). We also found suggestive linkage between the lean diabetic status and markers *APOA2–D1S484* (MLS = 3.04, *P* = .00018; MLB-LOD = 2.99, *P* = .00010) on chromosome 1q21-q24. Several other chromosomal regions showed indication of linkage with diabetic traits, including markers on chromosome 2p21-p16, 10q26, 20p, and 20q. These results (*a*) showed evidence for a novel susceptibility locus for type 2 diabetes in French whites on chromosome 3q27-qter and (*b*) confirmed the previously reported diabetes-susceptibility locus on chromosome 1q21-q24. Saturation on both chromosomes narrowed the regions of interest down to an interval of <7 cM.

Introduction

Type 2 diabetes (MIM 125853) is a heterogeneous disorder of glucose metabolism characterized by both insulin resistance and pancreatic β-cell dysfunction. The chronic hyperglycemia is often associated with essential hypertension, obesity, and dyslipidemia in a complex metabolic syndrome leading to severe vascular complications, including blindness, end-stage renal failure, and coronary-heart disease. The prevalence of type 2 diabetes in Westernized countries is 3%–10%, and epidemiological studies predict it may reach a total of 30%

in the next decade, which makes it a major health problem worldwide.

The familial clustering of type 2 diabetes, together with the higher concordance rates in MZ twins compared with DZ twins (Barnett et al. 1981; Hopper et al. 1999) and the close correspondence in hybrid populations, between genetic-admixture rates and disease prevalence (Knowler et al. 1988), suggest a genetic predisposition to type 2 diabetes, with a recurrence risk to first-degree relatives of ~3.5 (Rich 1990). In <10% of the cases, type 2 diabetes appears to segregate in a Mendelian fashion. However, in the majority of cases, the pattern of inheritance of the disease suggests a complex genetic interaction with environmental factors.

Considerable effort has been made worldwide during the last decade to identify the underlying genetic determinants. Studies of monogenic forms of type 2 diabetes led to the identification of several diabetogenes with major effects in rare families, including the insulin, insulin-receptor, and glucokinase genes and the four tran-

scription-factor genes encoding HNF1-α, HNF4-α, HNF1-β (hepatocyte nuclear factor), and IPF1 (insulin promoter factor 1), as well as mutations in the mitochondrial DNA (reviewed by Elbein et al. [1997]). However, these genes do not seem to play a major role in the more common forms of type 2 diabetes. Studies of candidate genes in pathways impaired in subjects with type 2 diabetes led to the identification of genes that may increase the risk for type 2 diabetes, including the *IRS1* (insulin receptor substrate 1), glucagon-receptor, glycogen synthase, sulfonylurea-receptor, and *IPF1* genes (Elbein et al. 1997). In any case, these genes contribute, to a modest or marginal extent, to genetic susceptibility to type 2 diabetes.

Several groups, therefore, have undertaken genomewide scans to identify loci for type 2 diabetes and related phenotypes. So far, genomewide significant linkages (Lander and Kruglyak 1995) for such susceptibility loci were identified on several distinct chromosomes in different populations. These include loci on chromosomes 2q37 (Hanis et al. 1996), 15q21 (Cox et al. 1999), 10q26 (Duggirala et al. 1999), and 3p (Ehm et al. 2000) in Mexican Americans; 12q24 (Mahtani et al. 1996) and 1q21-1q23 (Elbein et al. 1999) in whites; and 1q21-1q23 and 11q23-q25 in Pima Indians (Hanson et al. 1998). Overall, these results underscore the genetic heterogeneity of type 2 diabetes in diverse ethnic groups and the need of performing carefully designed wholegenome scans in other populations, either to identify new diabetes-susceptibility genes and/or to replicate previous findings.

To search for a major susceptibility locus (loci) contributing to type 2 diabetes in the French white population, we have completed a genomewide search for diabetes-related traits in 143 families with affected sib pairs. Here, we report evidence for a novel susceptibility locus for diabetes on chromosome 3q27-qter and independent confirmation of a locus on chromosome 1q21-q24. Subsequent fine-mapping in those regions has narrowed down the regions of interest to an interval of <7 cM.

Subjects

Families have been recruited since 1990 by means of a publicly advertised campaign for "200 families to overcome diabetes," and details of the recruitment have been described elsewhere (Vionnet et al. 1997). Among the 402 type 2–diabetes families collected with at least two affected sibs, 145 have been initially included in the study, according to criteria the aim of which was to reduce heterogeneity of the families and to maximize the power of the study. A family was included if there were at least two diabetic subjects in the sibship being treated for diabetes (to load the families with a severe form of type 2 diabetes) and at least three individuals (parents or additional sibs) available for the study (to assist the reconstruction of parental genotypes and to avoid nonfull sibs). Families presenting bilineal inheritance of type 2 diabetes or families with subjects diagnosed at age <25 years (i.e., potentially having the Maturity-Onset Diabetes of the Young [MODY] phenotype) or at age >65 years only (potential phenocopy caused by environmental factors rather than genetic ones) or families with mixture of type 1 and type 2 diabetes (potential Latent Autoimmune Diabetes of Adults [LADA]) were discarded. However, in one family, one individual was diagnosed at age 23 years and had positive anti-GAD antibodies. Nonwhite families or families with mutations in a known diabetes-susceptibility gene were also excluded from the study. Prior to genotyping of all the markers for the genome scan, 18 markers for the X chromosome (panel 28) were analyzed to check for DNA inconsistencies and Mendelian inheritance. From a preliminary selection of 145 families, 10 were discarded and were replaced by 12 additional families checked in the same manner. After the genome scan was completed, four families had to be discarded from the analyses because of recurrent Mendelian incompatibilities. All analysis results presented in this report were thus obtained in 143 families, corresponding to 148 nuclear families.

Phenotypes were determined in the light of the clinical report and the results of the latest measurements for fasting and/or oral glucose-tolerance test according to the recently established American Diabetes Association criteria for type 2 diabetes (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997): "diabetic" (DM) when receiving oral hypoglycemic agents or insulin ⩾1 year after the diagnosis, or when fasting glycemia was 7 mmol/l or glycemia 2 h after an oral glucose load was 11.1 mmol/l; "glucose intolerant" (GI) when fasting glycemia was 6.1–7.0 mmol/l or when 2-h glycemia was 7.8–11.1 mmol/l; "normoglycemic" (NG) when fasting glycemia was <6.10 mmol/l and 2-h glycemia was <7.8 mmol/l; "unknown" (UK) when no data were available or type 1 diabetes was present. Accordingly, the 637 individuals included in the study comprised 432 DM, 72 GI, 129 NG, and 4 UK subjects (tables 1 and 2). We designed six qualitative traits, taking into account the diabetic status, the age at diagnosis, and the body-mass index (BMI) of each subject in an attempt to stratify families into clinically more-homogeneous groups (table 3A). A maximum number of 677 affected sib pairs were analyzed with the affection status "large," and details of the family structure are given in table 3B for all six subgroups. Both parents were available in 6 families, and one parent was available in 30 more families.

Table 1

Clinical Characteristics of All Participants

Participant Status	Sex Ratio (M:F)	Age at Time of Study (years) ± SD (range)	Age at Diagnosis (years) ± SD (range)	Duration of Disease (years) ± SD (range)	BMI (kg/m^2) ± SD (range)
Nondiabetic ($n = 129$)	40:89	61.87 ± 12.69 (28–91)	...	...	25.1 ± 4.1 (14.98–36.71)
Diabetic ($n = 432$)	198:234	62.85 ± 9.6 (31–93)	49.5 ± 10.65 (23–83)	13.41 ± 9.8 (0–63)	27.89 ± 4.48 (15.11–45.91)
Glucose intolerant ($n = 72$)	36:36	61.15 ± 9.41 (41–87)	59.06 ± 9.56 (41–82)	2.08 ± 4.49 (0–24)	27.37 ± 4.70 (19.23–41.77)

Methods

Genotyping

Initial 10-cM genomewide screen.—Genomic DNA was isolated from leukocytes using the Puregene DNA Isolation Kit, according to the supplier's protocol (Gentra Systems). Genotyping was performed using a fluorescently labeled human linkage-mapping set (PE-LMSV2) comprising 400 highly informative microsatellite markers, with an average intermarker spacing of 9.7 cM. Multiplex PCR conditions were set up for each of the 28 panels in such a manner as to amplify the 400 markers in 87 PCRs. PCR (95°C for 12 min, followed by 30 cycles of 94°C for 15 s, 55°C for 15 s, 72°C for 30 s, and 72°C for 10 min) was performed on GeneAmp PCR system 9700 (Perkin-Elmer): 10-ml reactions; 40 ng of genomic DNA, 2.5 mM MgCl$_2$, 0.25 mM each dNTP (Pharmacia), a variable amount (0.2–1.5 pmol) of 5′ and 3′ primers, and 0.4 U Ampli*Taq* Gold DNA polymerase (Perkin Elmer) in 1 × PCR Buffer II (Perkin Elmer). (Multiplex PCR conditions are available from the authors on request.)

Pooled amplification products were electrophoresed through 5% polyacrylamide gels (Long Ranger Singel pack [Perkin Elmer]) for 1.5 h at 2,000 V on 24-cm plates on an ABI 377 DNA sequencer. An automated 96-channel pipettor Multimek 96 (Beckman) was used for all the pipetting steps. Semiautomated fragment sizing was performed by use of GENESCAN 3.0 software (ABI), followed by allele calling with GENOTYPER 2.1 software (ABI). Each genotype was reviewed independently by two members of the research team to confirm the accuracy of allele calling. Marker D14S68 was replaced by D14S67 because of reading difficulties. All markers but D12S310, the amplification of which failed, were included in the analysis. Two additional markers (D5S429 and D8S279) were added to the 399, to fill in gaps >20 cM. The overall data dropout rate for the 255,437 genotypes (401 × 637) analyzed was <4%. The average heterozygosity for the 401 markers used in the first-stage genome search was 0.79. Incompatibilities were searched for with the PED-CHECK 1.1 program (O'Connell and Weeks 1998) and inconsistencies resolved.

"Fine mapping" genotyping.—After the first-stage ge-nome search, we observed that linkage was primarily supported with markers on 3q27-qter and 1q21-q24 across most methods and phenotypes. We then saturated these regions with 16 additional genetic markers in each region, to confirm and further localize the linkage results. Each of the 32 markers was amplified individually by PCR, and genotyping was performed similarly to the genomewide scan. In addition, PCR and genotyping were performed a second time for the markers used in the genome scan and were localized in 3q27-qter and 1q21-q24 loci (seven and five markers, respectively). The overall data dropout rate for the 28,028 genotypes—16 × 2 additional + 12 "genome-scan" markers × 637 individuals—analyzed in this second step was of 0.5%, thus ensuring maximal informativity for multipoint analyses. The information content for markers used in the multipoint analyzes exceeded 0.85 for both regions.

Data Statistical Analysis

Nonparametric two-point and multipoint analyzes were performed with the programs MAPMAKER-SIBS 2.0 (MLS; Kruglyak et al. 1995), by using the "unweighted" option, and MLBGH 1.0 (Abel and Muller-Myhsok 1998), for autosomal markers. The maximum-likelihood binomial (MLB) method, which is based on the binomial distribution of parental marker alleles among affected offspring, overcomes the common problem of multiple sibs by considering the sibship as a whole. Two-point and multipoint analyzes of the chromosome X markers were performed using ASPEX (©1998 David A. Hinds and Neil Risch). Allele frequencies for the parameter file were generated from the data with DOWNFREQ 1.1 (Terwilliger et al. 1995).

Marker map positions for the genomewide scan and the high-resolution map on chromosome 3q27-qter were obtained from the sex-averaged maps compiled by Généthon and are given in Haldane centimorgans (cM). The high-resolution map order, from the Généthon map, of all markers tested at 3q27-qter was consistent with the Marshfield map order and with the results of mapping in the Genebridge 3 Radiation Hybrid Panel (Research Genetics) (data not shown). Of the 16 additional markers of chromosome 1q21-q24, 5 belonged to the Marshfield sex-averaged linkage map. For the high-resolution map on chromosome 1q21-q24, we used the

Table 2

Medication History of Diabetic Participants

Medication History	% of Diabetic Participants
Diet alone	10.7
Sulfonylureas (Sf)	24.9
Biguanides (Bg)	14.0
Insulin	12.6
Sf and/or Bg + insulin	5.3
Sf + Bg	30.9
Glucor or mediator	1.6

map positions from the Marshfield map, since mapping in the Genebridge 3 Radiation Hybrid Panel was more consistent with the Marshfield map order than with the Généthon map. Intermarker distances in our sample were systematically checked with a program derived from VITESSE (O'Connell and Weeks 1995).

When we restrict the sample to young-onset diabetes status, the sample size is reduced to 92 sib pairs, and we observe unequal sibship sizes. It is then legitimate to investigate the reliability of the P values. We simulated the transmission of the marker keeping the familial structure and affection status. When the founders had typed parents, we used their actual genotype and simulated the allele transmission. When they were unknown, their genotypes probability was determined according to the population allele frequency and their offspring genotypes. A total of 70,000 replicates were simulated through Monte Carlo method and were analyzed with MLBGH.

Results

First-Stage Genome Scan

Table 4 summarizes results of the multipoint analyses of the 401 framework markers from the initial genome scan. We considered any region with a Z_{norm} of 2.3 and a LOD score of 1.17 ($P = .01$) as "potentially interesting," and we applied the criteria of Lander and Kruglyak to further define regions of significant ($Z_{norm} = 4.11$, LOD $= 3.6$, $P = .00002$) or suggestive ($Z_{norm} = 3.19$, LOD $= 2.2$, $P = .0007$) linkage. Overall, 26 of the 2,298 tests using the MLB statistics for the autosomal chromosomes (383 markers tested for the six phenotypes) gave LOD scores ≥ 1.17 ($P \leq .01$), corresponding to 22 distinct markers that were distributed in 15 distinct genomic intervals (table 4A). The MLS statistics also gave LOD scores ≥ 1.17 ($P \leq .01$) for those genomic intervals (table 4A), as well as for markers in nine additional regions (table 4B). Of the 108 tests performed using the MLS statistics (with ASPEX program) for the X chromosome, 2 showed indication of linkage (table 4C). Most of the multipoint results were supported by

single-point analyzes (data not shown). There was a cluster of linked markers on chromosomes 3, 1, and 2. The positive chromosome 3 markers ($P = .01$) spanned >38 cM on the terminal q arm and gave positive results for two of six diabetes phenotypes with both statistics. Genomewide significance was observed at the *D3S1580* locus when the MLS statistic was used (MLS $= 3.91$, $P = .00002$ for the "large young-onset" phenotype), consistent with suggestive linkage using the MLB statistic (LOD $= 2.97$, $P = .00011$). Linkage was still suggestive with both statistical methods when applying a conservative correction factor for the number of traits studied by multiplying the P value by 6 (number of phenotypes). Linkage was also suggested in this chromosomal region with the "strict-young-onset" phenotype, with both MLS and MLB statistics: at *D3S1580*; MLS $= 2.89$, $P = .00026$, MLB-LOD $= 2.24$, $P = .00067$ (table 4A). When we restricted the sample to young-onset diabetes status, the sample size was reduced to 92 sib pairs, and we observed unequal sibship sizes. However, simulation studies for the chromosome 3 results showed that the empirical P values were very close to the value expected under asymptotic conditions. Therefore, we can rely on the P values given by the MLB method.

The positive chromosome 1 markers spanned >20 cM on the q arm and also gave positive results for two diabetes phenotypes. Suggestive linkage was observed with the "strict-lean" phenotype with both the MLS and MLB statistics, and results remained potentially interesting after Bonferroni correction. Positive results for chromosome 2 markers were primarily for the "strict" phenotype, with suggestion of linkage only with the MLB analyses, and spanned an interval of 26 cM on the p arm.

Second-Stage Mapping of Chromosomes 3q and 1q

From the first-stage scan, the strongest and most consistent evidences for linkage were found for markers from chromosomes 3q27-qter and 1q21-q24, across different analytical methods and different phenotypes. We thus added 16 more markers in the 3q27-qter and 1q21-q24 regions, for totals of 23 and 21 markers with average map densities of 2.6 and 1.9 cM, respectively. Figures 1A and 1B show results from the multipoint analyzes for both MLS and MLB statistics of the saturation of 3q with the "large young-onset" phenotype and 1q with the "strict-lean" phenotype. These analyzes further supported and strengthened the findings from the initial 10-cM scan. Evidence of linkage was confirmed in the 3q27-qter region with both the "large young-onset" and "strict young-onset" phenotypes (*D3S1580* ; MLS $= 4.67$ and 3.56 ($P = .000004$ and $.000054$, respectively) MLB-LOD $= 3.43$ and 2.65

Table 3

Description of Affection Status and Structure of the 148 Nuclear Families

3A. Description of Affection Status for the Six Phenotypic Groups[a]

Affection Status	Large	Strict	Large-Lean	Strict-Lean	Large Young-Onset	Strict Young-Onset
Affected	NIDDM+GI	NIDDM	(NIDDM+GI) with BMI <27	NIDDM with BMI <27	(NIDDM+GI) with age at onset <46	NIDDM with age at onset <46
Unknown	NA	NA+GI	NA+(NIDDM +GI) with BMI >27	NA+GI+ (NIDDM with BMI >27)	NA+(NIDDM+ GI) with age at onset >46	NA+GI+ (NIDDM with age at onset >46)
Nonaffected	NG	NG	NG	NG	NG	NG

3B. No. of Families with n Affected in Each Phenotypic Group[b]

n	Large	Strict	Large-Lean	Strict-Lean	Large Young-Onset	Strict Young-Onset
2	43	66	37	40	41	45
3	54	54	19	11	11	6
4	30	18	5	5	3	3
5	12	6	1			
6	4	1	3	1		
7	4	2				
8	1					
Total families	148	147	65	57	55	54
Total sib-pairs	677	453	179	113	92	81

[a] Affection status was determined taking into account patients' diabetic status (overt diabetes [NIDDM], GI, NG, or data not available [NA]), BMI (kg/m^2), and age at diagnosis of diabetes. Three affection statuses were used for the statistical analyses: affected, nonaffected, and unknown (coded 2, 1, and 0, respectively).

[b] Total number of sib pairs was calculated with the following formulae: (number of families with *n* affected, $n = [2–8]$) $X[nX(n − 1)]/2$.

($P = .00003$ and $.00024$, respectively). Moreover, the high-resolution mapping identified an indication of linkage, which was not observed at the 10-cM initial genome scan mapping phase, with an additional phenotype that is the "strict" phenotype (i.e., type 2 diabetes) regardless of the age at diagnosis (453 sib pairs, MLB-LOD = 1.28, $P = .007$).

On the other hand, although they did not reach the Lander-Kruglyak statistical thresholds for significance, results in the 1q21-q24 region were highly suggestive with the "strict-lean" phenotype (*APOA2–D1S484*; MLB-LOD = 2.99, $P = .00010$; MLS = 3.04, $P = .00018$). This saturation step with dense genetic map allowed us to restrict the 1-LOD-unit CI (Ott 1991) from 11 to 6.2 cM and from 19 to 6.5 cM for the loci on chromosomes 3 and 1, respectively.

Discussion

The present genome scan in French whites provides (1) strong evidence for a susceptibility locus for diabetes and impaired glucose tolerance with early onset (at age <46 years) on chromosome 3q27-qter and (2) suggestive evidence that there is a diabetes-susceptibility gene in French whites in a previously reported region on chromosome 1q21-q24. In our genomewide-scan design for type 2 diabetes–susceptibility genes, emphasis has been focused on power to maximize the chances for success.

We used a large number of sib pairs (>200 for the "large" and "strict" phenotypes), and a 10-cM marker grid in a one-stage study yielding a power of >98% for a risk ratio (λ_s) of 3 and of >70% for a risk ratio (λ_s) of 2 (Holmans and Craddock 1997). Because genotyping errors lead to false-positive or false-negative results, automation and stringent quality-control criteria have been applied. Families were selected from among our large collection to be as homogeneous as possible, to avoid background noise caused by bilineal inheritance of the trait (DeStefano et al. 1998), other genetic forms of type 2 and/or type 1 diabetes (such as MODY or LADA), and nongenetic forms caused by environmental factors. Only families in which accurate inference of relationships among sib pairs was possible were included, to avoid reduced power to detect linkage caused by misclassification of half-sib pairs or unrelated individuals as full sibs (Boehnke and Cox 1997). Two powerful sib pair–based methods were used for analyses: the MLS method, which was previously shown to be most sensitive (Davis and Weeks 1997), and the MLB method, which takes into account sibships with several affecteds in a natural way and was shown to perform well in terms of type 1 error and power (Abel and Muller-Myhsok 1998; Abel et al. 1998).

Our best linkage result was found between markers on chromosome 3q27-qter and diabetes and impaired glucose tolerance diagnosed at age <45 years. Criteria

Table 4

Multipoint Results of the 10-cM Genome-Scan Initial Stage

4A. Results of MLB and MLS Multipoint Analyses for Six Diabetes-Related Phenotypes

Interval[a]	Positive Markers[b]	Position[c] (cM)	Phenotype	MLB-LOD (P)	MLS (P)
1p21	D1S206	141.12	Strict-lean	1.27 (.00777)	1.48 (.00782)
1q21-q24	D1S484, D1S498	178	Strict-lean	2.47 (.00037)	2.50 (.00066)
	D1S484	178	Strict	1.23 (.00877)	1.12 (.01911)
1q43	D1S2785	276.23	Large young-onset	1.39 (.00575)	1.64 (.00533)
2p25	D2S319	6.17	Strict young-onset	1.61 (.00324)	2.02 (.00212)
2p21-p16	D2S2259, D2S391	75.31	Strict	2.28 (.0006)	2.27 (.00116)
2q24-q23	D2S2330	179.22	Large-lean	1.25 (.00826)	1.22 (.01507)
3q27-qter	D3S1580, D3S1262, D3S1601	217.97	Large young-onset	2.97 (.00011)	3.91 (.00002)
	D3S1580, D3S1601	217.97	Strict young-onset	2.24 (.00067)	2.89 (.00026)
4p16	D4S2935, D4S412	12.5	Large	1.34 (.00643)	1.35 (.01087)
4q31	D4S1539	185.99	Large-lean	1.87 (.00166)	2.09 (.00175)
5q31-q33	D5S410, D5S436	159.43	Large	1.52 (.00412)	1.67 (.00492)
7q11	D7S669	92.74	Large-lean	1.32 (.00682)	1.68 (.00479)
9q34	D9S158	160.01	Strict-lean	1.30 (.00724)	1.22 (.01494)
10q26	D10S1655	174.65	Strict	1.59 (.00342)	1.24 (.01427)
	D10S212	185.23	Large	1.22 (.00899)	1.69 (.00472)
	D10S212	185.23	Large-lean	1.44 (.00504)	1.45 (.00841)
11p15	D11S1338	15.24	Strict young-onset	1.34 (.00648)	1.25 (.01371)
19p13	D19S221	36.92	Large young-onset	1.20 (.00948)	1.26 (.01358)

4B. Additional Loci with MLS Score >1.17

Interval[a]	Positive Markers[b]	Position[c] (cM)	Phenotype	MLB-LOD (P)	MLS (P)
2p25	D2S168	29.3	Strict	.82 (.02621)	1.90 (.00278)
2p23	D2S305	41.64	Large	1.16 (.0103)	1.98 (.0023)
	D2S305	41.64	Large young-onset	.62 (.04537)	1.60 (.00581)
	D2S305	41.64	Strict young-onset	.80 (.02707)	1.70 (.00459)
8q22	D8S270	104.72	Large	.97 (.01723)	1.44 (.00865)
12p11	D12S345	55.56	Large young-onset	.84 (.02447)	1.41 (.00925)
15q15	D15S1007	26.78	Strict young-onset	.74 (.03211)	1.50 (.00753)
16p11	D16S3068	14.6	Strict young-onset	1.09 (.01253)	1.39 (.00971)
18q12	D18S478	54.71	Large young-onset	1.09 (.0126)	1.75 (.00401)
20p13-p12	D20S186	33.93	Large young-onset	1.14 (.01093)	1.86 (.00312)
	D20S186, D20S115	33.93	Strict young-onset	1.08 (.01304)	1.68 (.00475)
20q13	D20S178	66.95	Large	.05 (.06295)	1.72 (.00432)

4C. Results of ASPEX Multipoint Analyses for Six Phenotypes

Interval[a]	Positive Markers[b]	Position[c] (cM)	Phenotype	MLB-LOD (P)	MLS (P)
Xp11-p21	DXS991	89.34	Large		1.67 (.00491)
	DXS991	89.34	Strict		1.56 (.00643)

[a] Chromosomes locations were determined from the map available in the Genome Database and the CEDAR database.

[b] When several markers per line are shown, the first one is the nearest to the top of the peak.

[c] Map positions of the nearest marker to the top of the peak, obtained from sex-averaged maps compiled by Généthon.

for assessment of statistical significance in genetic-linkage studies of complex traits have been controversial. Specific critical values have been proposed; some investigators have suggested that a relatively stringent threshold of $Z > 3.6$ is needed to obtain a genomewide $P < .05$ (Lander and Kruglyak 1995), whereas others have maintained that the less stringent traditional criterion of $Z > 3.0$ is unlikely to be a false positive (Holmans et al. 1997; Morton 1998; Rao 1998). In addition,

when several correlated traits have been analyzed, it is unclear how to adjust for multiple comparisons. The present analysis provides strong evidence for linkage on chromosome 3 with diabetes and impaired glucose tolerance diagnosed at age <45 years ($Z = 4.67$) and for diabetes diagnosed at age <45 years ($Z = 3.56$). Both two-point and multipoint MLS and MLB statistics gave consistent results that were still significant after Bonferroni correction for the number of phenotypes ana-
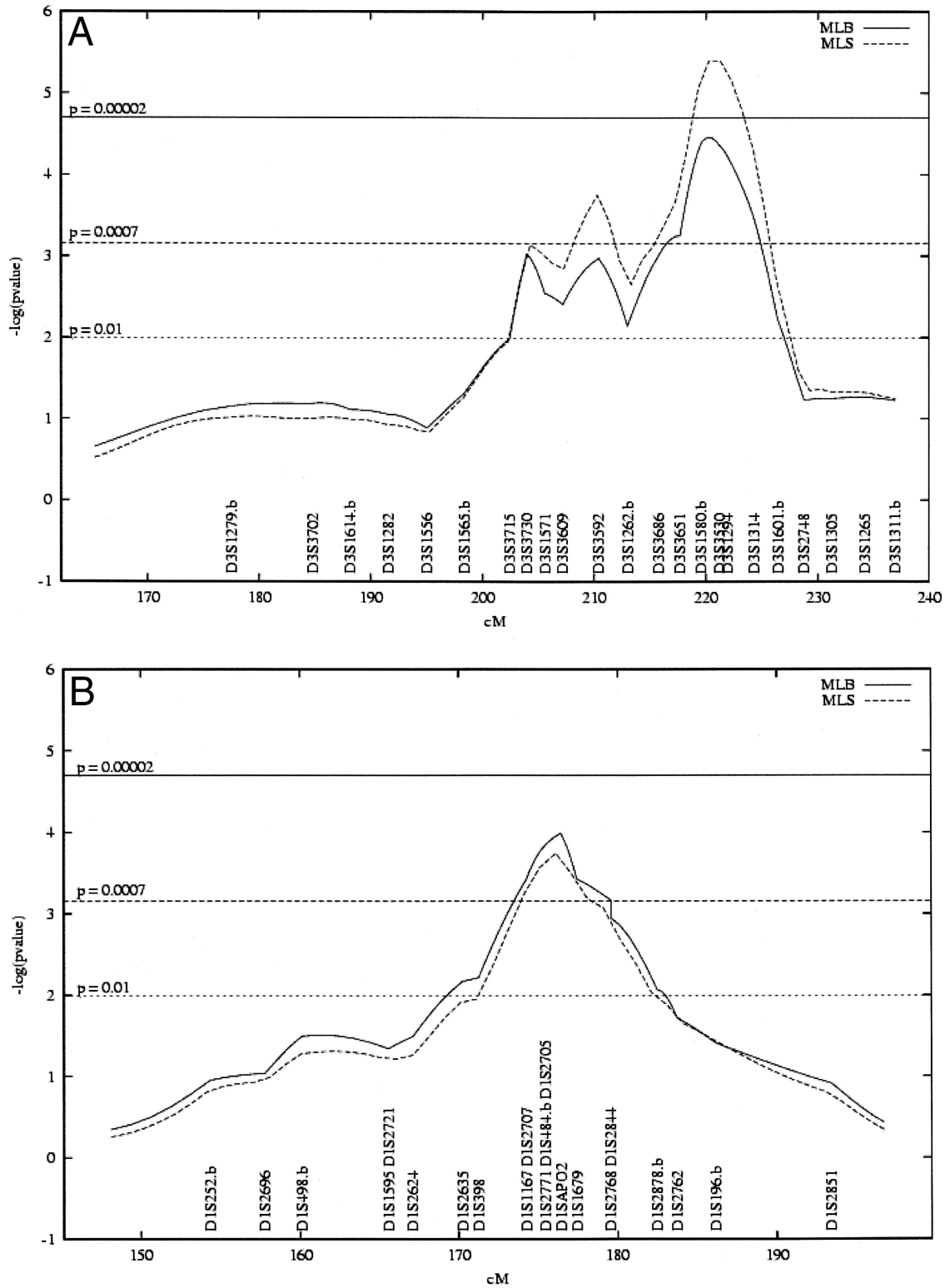
Figure 1   Results of the MLB and MLS multipoint analyses for the high-resolution genetic mapping at chromosome 3q27-qter with the "large young-onset" phenotype (*A*) and at chromosome 1q21-q24 with the "strict-lean" phenotype (*B*). The thresholds for putative (LOD 1.17; $P = .01$), suggestive (LOD 2.2; $P = .0007$), and significant linkage (LOD 3.6; $P = .00002$) are indicated by horizontal lines. Marker names followed by ".b" were also used in the first stage 10-cM genomewide screen and were fully retyped during the high-resolution mapping step (see Methods section). The horizontal axis corresponds to the partial genetic map of chromosomes 3q27-qter and 1q21-q24 intervals studied in the saturation step with high-density genetic maps. The vertical axis indicates the $-\log(P)$ obtained with multipoint analyses at each point in the studied interval.

lyzed. In addition, the width of the peak on chromosome 3q27-qter, given by all positions providing a P value of .01 in the multipoint analysis, was broad (>20 cM), which is an argument in favor of this peak being a true positive rather than a false positive (Terwilliger et al. 1997). We found linkage on chromosome 3 through stratification of families in clinically more-homogeneous subgroups, which is known to help in identification of susceptibility loci for a complex disease (Rao 1998). The result on chromosome 3q has been observed in a subgroup of 55 non-MODY families (37% of total families) with glucose intolerance or diabetes diagnosed between age 25 and 45 years, although both parents were not affected. Therefore, this group probably represents the most genetic form of non-MODY type 2 diabetes. Results of simulation studies showed that the nominal P values obtained in this reduced sample were reliable. On the basis of a single sample, it is difficult to unambiguously distinguish true from false linkage results. However, linkage of diabetes to the chromosome 3q27-qter region is supported by (1) the consistency of results across phenotypes and analytical methods, (2) the extremely low P values, and (3) the results of our simulations. Additional support comes from replication of results either in the same population or in other populations. We could not perform a replication study in our population, because the huge size of the replication sample recommended by simulation studies (Risch and Merikangas 1996) was incompatible with the small number of similarly ascertained families remaining in our French collection. Our region of positive linkage on chromosome 3q27-qter spanned >20 cM in the first-stage genome scan and was refined to <7 cM after the fine-mapping step was conducted. This region includes several reports of positive linkage with metabolic traits; we agree with Neel and colleagues (1998) that "type 2 diabetes, essential hypertension, and obesity [are] syndromes of impaired genetic homeostasis." Hegele et al. (1999) found an indication of linkage between *D3S2418* and type 2 diabetes in a 20-cM genomewide scan in a Canadian Oji-Cree population ($P = .01$). They did not find evidence of linkage disequilibrium between *D3S2418* and diabetes; neither did we with *D3S1580* in the 55 linked families using the Monte Carlo–based χ^2 implemented in CLUMP (Sham and Curtis 1995; data not shown). QTLs for impaired acute insulin response, insulin resistance in nondiabetics (Pratley et al. 1998) and in diabetics (Hanson et al. 1998), and waist:hip ratio (a measure for obesity) (Norman et al. 1998) were also suggested in this chromosomal region, in the sole Pima Indian population. Interestingly, a QTL for small low-density lipoprotein–particle phenotype, known to be a risk factor for cardiovascular disease, has been recently mapped in the same region on chromosome 3q27-qter (Rainwater et

al. 1999). Several studies have reported a polymorphism in the apolipoprotein D (*APOD*) locus, in the region of the maximal peak of our linkage at this chromosomal region, to be associated with diabetes and obesity (Hitman et al. 1992; Vijayaraghavan et al. 1994). Although none of those previously reported linkages on chromosome 3 reached statistical significance, as did the linkages in our study, they do strengthen our results, and it is tempting to speculate that the same locus is responsible for these results.

Saturation of the chromosome 3q27-qter region in our families reduced the 1-LOD-unit confidence interval (CI) to 6.2 cM around *D3S1580*, which, according to the Unified Database for Human Genome Mapping, spans ~4.5 mb, an interval of suitable size to initiate positional cloning and disequilibrium mapping efforts. This chromosomal region contains several potentially interesting genes, including the genes coding for somatostatin, apolipoprotein D, and inhibitor 2 of the synthase-activating enzyme, type 1 protein phosphatase, I-2 (PPP1R2), and the bifunctional enzyme (with enoyl-CoA-hydratase and 3-hydroxyacyl-CoA dehydrogenase activity) (MIM 182450, MIM 107740, MIM 601792, and MIM 261515, respectively).

We also found evidence of linkage between markers on chromosome 1q21-q24 and diabetes in lean patients (with BMI <27). Although not reaching the stringent statistical thresholds for significance, results on chromosome 1q were highly suggestive ($P = .00010$), and, again, the broad width of the peak in the initial genome scan (>20 cM) also suggested true-positive results. Linkage on chromosome 1q was also found through stratification of families, in a homogeneous subgroup of 57 families (38% of families) in which diabetes is enriched in lean patients. Since obesity is frequently associated with diabetes and is genetically determined as well, it could be a confounding variable in genetic studies of diabetes. Therefore, genetic studies of lean diabetic patients are more likely to identify true diabetes-susceptibility loci, rather than obesity-susceptibility loci. Our results on chromosome 1q confirm (and replicate) previous reports of evidence for linkage between diabetes and markers in the same region in at least two different populations. Elbein et al. (1999) reported evidence of linkage between diabetes and markers CRP/APOA2 in 19 multigenerational families of northern European ancestry from Utah using a recessive model for parametric analyzes. In a study of the Pima Indians, among whom diabetes and obesity are highly prevalent, Hanson et al. (1998) found evidence of a diabetes locus on chromosome 1q, a region that was not linked to obesity in their study. They observed a very strong evidence for linkage with diabetes ($Z = 4.1$) at *D1S2127,* in the 55 sib pairs who had onset of diabetes at age <25 years, supported by comparisons of sib pairs concordant for diabetes

versus discordant sib pairs at *D1S1677*. The finding of linkage in three different populations strengthens the evidence for a diabetes-susceptibility locus in this region.

Saturation of the chromosome 1q21-q24 region in our families reduced the 1-LOD-unit CI to 6.5 cM around *APOA2/CRP*, which, according to the Unified Database for Human Genome Mapping, corresponds to ~6 mb. Indeed, positional cloning and disequilibrium-mapping efforts have already been initiated in diabetic Pima Indians to identify the chromosome 1q locus, and several of the potential candidate genes have been screened (Baier et al. 1998; Wolford et al. 1999*a*). These identified a linkage disequilibrium between diabetes and single-nucleotide polymorphisms near the *KCNJ9* gene coding for the G-protein-coupled inward rectifier K(+) channel homolog *GIRK3*, suggesting that the diabetes-susceptibility gene on chromosome 1q21-q24 is closely linked to the *KCNJ9* locus (Wolford et al. 1999*b*). Linkage disequilibrium between diabetes and chromosome 1 markers in the same region was also found in the Old Order Amish (St. Jean 1999*a*, 1999*b*). The possible role for those genes in the French families that contributed to our linkage results is under investigation.

We identified 22 other regions showing at least nominal indication of linkage ($P < .01$). When looking very closely at the published data, we found similar results in other studies in most of our regions. Of particular interest is our linkage result on chromosome 10q26 that largely overlaps the region of linkage with diabetes and age at onset of diabetes identified in Mexican Americans (Duggirala et al. 1999). We were therefore able to replicate their findings independently in our population.

Our suggestive linkage results on chromosome 2p21-p16 with the diabetic phenotype are supported as well by several reports for metabolic traits in that region, mostly obesity-related phenotypes, including QTLs for leptin levels, fat mass, or BMI in different populations (Comuzzie et al. 1997; Hager et al. 1998; Rotimi et al. 1999). It is therefore possible that a genetic defect localized on chromosome 2p determines the occurrence of both obesity and type 2 diabetes. It has already been reported that some loci, such as the one on chromosome 11q23-q25, harbor diabetes and obesity genes (Hanson et al. 1998).

As far as chromosome 20 is concerned, we were the first to report positive linkage on chromosome 20q in another selection of French diabetic families (Hani et al. 1996; Zouali et al. 1997). Afterward, several studies confirmed our findings (Bowden et al. 1997; Ji et al. 1997; Ghosh et al. 1999), including results of a meta-analysis of all data available worldwide for this chromosome (Boehnke et al. 1998). Obesity-related traits have also been mapped on this chromosome (Lembertas

et al. 1997; Norman et al. 1998; Lee et al. 1999). From our studies and others, it seems possible that there are two diabetes- and/or obesity-susceptibility genes on chromosomes 20p and 20q. Further fine mapping and sequencing of selected candidates (*HNF3-β, MC3R, CEBP-β,* and *Nkx2.2*) are under way to elucidate the role of chromosome 20 genes in the French diabetics.

Because emphasis has been placed on power in our genomewide-scan design for type 2 diabetes–susceptibility genes, in this study, we were able to exclude linkage at the diabetes loci on chromosomes 2q37, 15q21, and 12q24—identified in Mexican-American and Finnish subjects, respectively—as well as at the diabetes-and-obesity locus on chromosome 11q23-q25 that was identified in Pima Indians or the diabetes locus recently mapped on chromosome 3p in Mexican Americans. It is therefore very likely that genes that play an important role in isolated or recently admixed populations have a marginal role, if any, in a representative admixed population such as these French whites.

In conclusion, we report the first evidence for a novel diabetes-susceptibility locus gene on chromosome 3q27-qter. Several results of positive, although not significant, linkage at this locus that were previously reported in the literature support our findings. In addition, we independently confirm linkage with diabetes in the 1q21-q24 interval (Hanson et al. 1998; Elbein et al. 1999). Other loci on chromosomes 2p21-p16, 10q26, and 20 are potentially interesting but await further studies to be confirmed.

Acknowledgments

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

CEDAR, http://cedar.genetics.soton.ac.uk/pub (for chromosome locations)
Généthon, ftp://ftp.genethon.fr/pub/Gmap/Nature-1995 (for marker maps)
Genome Database, http://www.gdb.org (for chromosome locations)
Marshfield, http://research.marshfieldclinic.org/genetics/ (for marker map)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for type 2 diabetes [MIM 125853], somatostatin [MIM 182450], apolipoprotein D

[MIM 107740], type 1 protein phosphatase I-2 [PPP1R2; MIM 601792], bifunctional enzyme with enoyl-CoA-hydratase and 3-hydroxyacyl-CoA dehydrogenase activity [MIM 261515])

Unified Database for Human Genome Mapping, http://bioinformatics.weizmann.ac.il/udb/

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