Brief Genetics Report

Linkage but Not Association of Calpain-10 to Type 2 Diabetes Replicated in Northern Sweden

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We present data from a genome-wide scan identifying genetic factors conferring susceptibility to type 2 diabetes. The linkage analysis was based on 59 families from northern Sweden, consisting of a total of 129 cases of type 2 diabetes and 19 individuals with impaired glucose tolerance. Model-free linkage analysis revealed a maximum multipoint logarithm of odds score of 3.19 for D2S2987 at 267.7 cM ($P = 0.00058$), suggesting that a gene conferring susceptibility to type 2 diabetes in the northern Swedish population resides in the 2q37 region. These data replicate, in a European population, previously identified linkage of marker loci in this region to type 2 diabetes in Mexican Americans. In contrast, no evidence in support of association to the previously identified single nucleotide polymorphisms in the calpain-10 gene was observed in a case-control cohort derived from the same population. *Diabetes* 55:1879–1883, 2006

Type 2 diabetes accounts for the majority of diabetes cases and affects a significant proportion of the adult population worldwide. Despite extensive linkage and association studies to identify genes that contribute susceptibility to this disease, the nature of these factors remains largely elusive. A previous study identified linkage between type 2 diabetes and a region on 2q36-37 (1). This was followed by an association study concluding that increased risk for type 2 diabetes was associated with a certain combination of single nucleotide polymorphism (SNP) haplotypes in the calpain-10 gene located on 2q37 (2). The combination of two haplotypes identified in the SNPs UCSNP-43, -19, and -63 significantly increased the risk for type 2 diabetes, while no increase in risk was recorded for homozygotes in either of the "risk haplotypes." These results suggested an interaction between the two haplotypes, leading to increased risk of developing disease. Follow-up investigations have been inconsistent (3–9). One study from the U.K. showed association to another SNP in the calpain-10 region (UCSNP-44) but not to the three polymorphisms originally identified (7). We here report replication of linkage between markers in this chromosomal region to type 2 diabetes in families derived from northern Sweden.

RESEARCH DESIGN AND METHODS

A population-based register of individuals with type 2 diabetes was used to identify probands aged 30–60 years. First-degree relatives with diabetes were identified, and diabetes diagnoses were confirmed by scrutinizing medical records regarding symptoms and blood glucose measurements, following 1999 World Health Organization criteria (10). Validation of type 2 diabetes diagnosis was based on present medication and analysis of C-peptide levels and aided by a validated algorithm based on BMI at onset and duration of time until the start of insulin therapy, if any (11). To exclude late-onset autoimmune diabetes in adults, IA-2 and GAD antibodies were analyzed. Mutations in the coding regions of maturity-onset diabetes of the young genes 1–4 were excluded by high-performance liquid chromatography analysis. Moreover, no indications that any of the patients in the study had impaired hearing were found, arguing against diabetes-associated mitochondrial mutations.

A total of 59 families were investigated, including 117 clinically diagnosed patients with type 2 diabetes and 114 adult relatives with no prior record of diabetes. A 75-g oral glucose tolerance test (OGTT) was performed on 84 of the adult relatives without known diabetes. Glucose tolerance was classified according to World Health Organization criteria (10). HbA_{1c} was analyzed by high-performance liquid chromatography with a normal range of 3.9–5.3%. The normal range for fasting serum C-peptide was 370–1,470 pmol/l. This analysis revealed 12 individuals with previously undiagnosed diabetes from the OGTT and 19 individuals with impaired glucose tolerance (IGT), leaving 71 unaffected and 12 untested relatives.

In disease model 1, individuals with IGT, likely to represent a state of pre-diabetes, as well as patients with a diabetic OGTT or clinically diagnosed type 2 diabetes were set as affected. This model reflects a scenario where type 2 diabetes is only an extreme case of insulin resistance, and those with IGT are expected to develop overt disease or at least carry many of the potential genetic factors involved with type 2 diabetes. In disease model 2, only patients with a diabetic OGTT and overt type 2 diabetes were set as affected, IGT patients were set as unknown. Model 2 should better represent a situation in which the population is exposed to a sedimentary lifestyle with meals dominated by sugars and fat and only genetically susceptible individuals go on to develop type 2 diabetes.

For association studies, an independent case-control cohort including 872 case subjects with clinically diagnosed type 2 diabetes and 857 control subjects matched with respect to age, sex, and geographical origin was used. Samples were obtained from the Medical Biobank at Umeå University.

Genome-wide linkage analysis. Genomic DNA was prepared from whole blood using standard phenol-chisam methods and analyzed using the ABI linkage panel set with an average spacing of 10 cM (ABI PRISM Linkage Mapping Set v2.5MD10; Applied Biosystems, Foster City, CA). PCR products were analyzed on ABI PRISM 3100 or 3730 DNA sequencers and genotypes analyzed using GeneMapper 3.7 (Applied Biosystems). The largest gap in the genome-wide scan was 14.7 cM.

Additional markers from the ABI PRISM Linkage Mapping Set 5 cM and markers ordered from DNA Technology (Aarhus, Denmark) were typed in the 2q37 region. A number of SNPs were typed to fill gaps where no informative

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Received for publication 14 November 2005 and accepted in revised form 27 February 2006.

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Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

DOI: 10.2337/db05-1495

TABLE 1
Clinical characteristics of the individuals included in the genome-wide scan

	Unaffected or no OGTT	IGT	Newly diagnosed type 2 diabetes*	Type 2 diabetes
n (%)	83 (35.9)	19 (8.2)	12 (5.2)	117 (50.7)
Men (%)	41 (35.4)	12 (10.3)	5 (4.3)	58 (50.0)
Women (%)	42 (36.5)	7 (6.1)	7 (6.1)	59 (51.3)
Median current age (years)	54	62	64	59
Median age at diagnosis (years)				50
Median BMI (kg/m^2)	26.9	29.6	29.4	30.2
Median BMI at diagnosis (kg/m^2)				30.3
Median C-peptide (pmol/l)	770	811	764	1,520
Median HbA_{1c} (%)	4.6	4.6	5.4	6.6

Data are *n* (%) unless otherwise indicated. *Newly diagnosed type 2 diabetes at OGTT.

microsatellite markers were available. The final average intermarker distance in the 2q37 region was 1 cM.

SNP genotyping. Four previously studied bi-allelic polymorphisms in calpain-10 were analyzed for association to type 2 diabetes. UCSNP-44, -43, and -63 were analyzed using TaqMan 7900HT SNP analysis. Assay-by-design assays were obtained from Applied Biosystems and analyzed according to the manufacturers instructions. UCSNP-19 was PCR amplified using standard protocols and run on an ABI PRISM 3100 DNA sequencer. Genotypes were analyzed using GeneMapper 3.0 (Applied Biosystems) and checked for inconsistencies using the PedCheck program (12).

Statistical analysis. Model-free multipoint linkage analysis was performed using the exponential model and the s_{pairs} scoring function within the computer program Allegro (13). Allele frequencies were estimated from all genotyped individuals with Merlin (14). The *P* values reported were computed by comparing the observed allele-sharing logarithm of odds (LOD) score with its complete data distribution and are not corrected for multiple testing. Linkage disequilibrium and haplotype analysis for markers in calpain-10 was performed with the program Haploview (15). Estimations of haplotype combinations in the control group were performed by the use of estimated haplotype frequencies and under the assumption of Hardy-Weinberg equilibrium. Among the case subjects, individual haplotype combinations were manually assigned according to the SNP genotype data. The assignment could be done unambiguously for 794 individuals.

To test for association of genotypes and type 2 diabetes in the case-control material, genotype-based odds ratios were calculated with their respective 95% CIs, using logistic regression. Individuals homozygous for the most common allele were set as the reference. To adjust for possible confounding effects, a stratification variable, based on the age, sex, and geographical area, was added into the regression model. These calculations were performed using the statistical software package SPSS, version 11.5. Association analysis in the family material was performed by TRANSMIT (16), a generalized transmission disequilibrium test program that handles missing parental data and uses information from multiple affected individuals within a family, even in the presence of linkage.

RESULTS AND DISCUSSION

The clinical and phenotypical characteristics of the individuals included in the genome-wide scan are summarized in Table 1. The clinically diagnosed individuals were obese (median BMI 30.2 kg/m^2) and had an early diagnosis of diabetes (median age at diagnosis 50 years). Their median C-peptide level was in the upper range (1,520 pmol/l). Of the 84 OGTT tests performed on "unaffected" relatives, 12 showed diabetic values and 19 indicated impaired glucose tolerance. In 30 subjects, no OGTT was performed; however, fasting or nonfasting glucose values were available for 10 and 8 subjects, respectively, of the 30, and these values were all within normal range.

Genome-wide allele-sharing multipoint linkage results are shown in Fig. 1. The initial linkage analysis of the family-based dataset using marker loci with an average intermarker distance of 10 cM revealed eight peaks with an allele-sharing LOD score >1.0 for either model 1 or 2 or both (at 78.1 and 252.7 cM on chromosome 2, at 66.6 cM on chromosome 3, at 30.2 cM on chromosome 7, at 11.0 cM and 51.8 cM on chromosome 11, at 148.3 cM on chromosome 12, and at 47.0 cM on chromosome 12). One of these

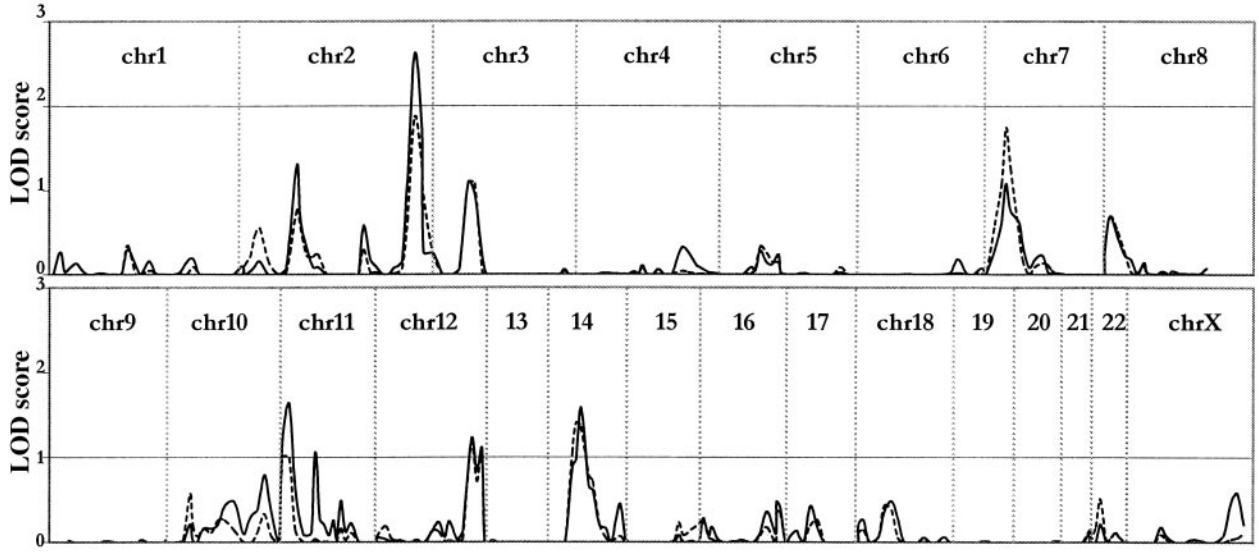

FIG. 1. Genome-wide multipoint allele-sharing LOD scores. Vertical axis denotes LOD scores; horizontal axis denotes relative centimorgan position on each chromosome (chr) (Genethon map). Solid lines, disease model 1; dashed lines, disease model 2.

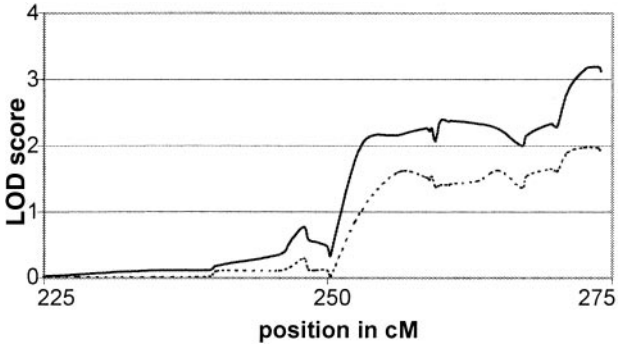

FIG. 2. Multipoint allele-sharing LOD scores for the linkage peak on 2q37. Solid line, disease model 1; dashed line, disease model 2.

regions, at 252.2 cM on chromosome 2, yielded a multipoint allele-sharing LOD score of 2.6 ($P = 0.0024$) at marker D2S338 when using model 1 including the IGT as affected. Setting only type 2 diabetes and OGTT as affected (model 2), the corresponding LOD score was found to be 1.87. This region contains calpain-10, identified in a previous linkage study as conferring susceptibility to type 2 diabetes (1). The four previously reported polymorphisms in the calpain-10 gene, UCSNP-44, -43, -19, and -63 (1), were found to be in strong linkage disequilibrium, with D′ values of 1 or close to 1. For this reason, only the most informative SNP, UCSNP-19, was included in the linkage calculation. As a result of this increased resolution map, the maximum allele-sharing LOD score on 2q37 increased to 3.19 ($P = 0.00058$) at 267.7 cM in marker D2S2987 for model 1, increasing to 1.97 for model 2 (Fig. 2). This data replicates the linkage results described by Hanis et al. (2), reinforcing the calpain-10 gene as a likely candidate for the type 2 diabetes susceptibility gene in the 2q37 region.

The four SNPs analyzed in the calpain-10 gene were further analyzed. Looking at the frequency of particular alleles of the SNPs, we found that the values of the square of the correlation between these four polymorphisms (R^2) were substantially lower than those of D′ (ranging from 0.020 to 0.358), suggesting that all markers were contributing information; therefore, they were all included in the construction of haplotypes. Haplotype analysis in the families contributing to the positive linkage at 2q37 did not reveal an overrepresentation of the previously reported 1-2-1/1-1-2 at-risk haplotype combination or an overrepresentation of the two haplotypes separately (data not shown).

We present data replicating evidence of linkage between type 2 diabetes and the 2q37 region in families from northern Sweden, reinforcing that the calpain-10 gene, located in this chromosomal region, contributes to the risk for type 2 diabetes also in this population. To our knowledge, this constitutes the first replication of linkage between type 2 diabetes and calpain-10, previously reported from studies of a Mexican-American population (1). The relative strength of the linkage on 2q37 to type 2 diabetes in the family material supports previous reports that isolated populations, such as the one in northern Sweden, may be more genetically homogeneous than more out-bred populations and thus especially useful for mapping of complex traits. We have previously demonstrated that linkage analysis of familial forms of complex diseases is a feasible approach in the population of northern Sweden (17), and the data presented here further support this. The subjects included in this study were selected for an early diagnosis of type 2 diabetes to increase the impact of genetic influence versus lifestyle factors. Their age and phenotype are consistent with the largest clinical intervention study in type 2 diabetes, the U.K. Prospective Diabetes Study (18), and representative of the clinical spectrum of the disease in this region. Both the diagnosis of diabetes and the classification of type 2 diabetes have been carefully validated. By performing an OGTT in almost all "unaffected" relatives, we have been able to define the spectra of glucose tolerance and identify previously unknown cases of type 2 diabetes and IGT.

While not reaching significant levels, the observed linkage could lend support to previous reports of linkage on chromosomes 3 (19,20) and 7 (21,22). The two peaks of linkage on chromosome 11, the peak on 11p15 containing the insulin gene and the peak in 11p12-p11 previously reported (21,23), have also been previously reported to be involved in type 2 diabetes. Our linkage peak on chromosome 12 contains the MODY3 gene, previously shown to be involved in type 2 diabetes (24). The linkage we found on chromosome 14 overlaps with previous reports (25,26), arguing that the same genetic factors may be involved in type 2 diabetes in the three studies.

We looked at association of type 2 diabetes to the four previously described SNPs within the calpain-10 gene using a large case-control cohort from northern Sweden. Power analysis of the material can be found in supplementary Tables 1 and 2 in the online appendix (available at http://diabetes.diabetesjournals.org). A study based on Danish and Swedish samples (8) found no association of the 1-1-2/1-2-1 haplotype combination to type 2 diabetes, perhaps due to the low frequency of the 1-1-2 haplotype in the Danish population (0.07 vs. 0.23 in Mexican Americans) (8). We obtained similar results in our population, where the frequency of the 1-1-2 haplotype is ~0.09 (Table 2). The frequency of the 1-2-1/1-1-2 risk haplotype combinations is estimated to be 0.06 in both case and control subjects. Moreover, transmission disequilibrium test analysis of all the four calpain-10 SNPs as well as their haplotypes, in the family material, did not provide any significant evidence in favor of association (data not shown). The fact that we do not see association with any of the four SNPs in our large and robust case-control material, with or without adjustments for age, sex, or geographical area and no association to particular haplotypes or haplotype combinations, suggests to us that these four polymorphisms are probably not responsible for increased risk of type 2 diabetes in northern Sweden. This is also in agreement with a recent meta-analysis made for population- and family-based association studies of calpain-10 to type 2 diabetes (27). It also suggests that the linkage to 2q37 will be explained by other polymorphic variants in or around calpain-10 or by genetic variation linked to another gene in close proximity.

ACKNOWLEDGMENTS

This work was supported by grants from the Kempe Foundation and by the Swedish Research Council-M.

The authors thank Pia Osterman and Ann-Charloth Nilsson at the Genotyping Core Facility, Umeå University, for excellent technical assistance and Dr. Lars Weinehall, who was responsible for the Västerbotten intervention program.

TABLE 2
Genotype frequencies, logistic regression analysis, and haplotype frequency estimations for UCSNP-43, -44, -19, and -63 in calpain-10

SNP	Genotype	Case subjects (%)	Control subjects (%)	OR (95% CI)	*P*
SNP-44	22	550 (63.1)	569 (66.4)	Ref.	
	21	285 (32.7)	255 (29.8)	0.86 (0.70–1.06)	0.152
	11	37 (4.2)	33 (3.8)	1.00 (0.61–1.66)	0.986
SNP-43	11	445 (51.6)	449 (52.1)	Ref.	
	12	361 (41.8)	342 (39.7)	1.07 (0.87–1.30)	0.535
	22	57 (6.6)	71 (8.2)	0.80 (0.55–1.17)	0.251
SNP-19	22	258 (33.2)	271 (35.0)	Ref.	
	12	395 (50.8)	400 (51.7)	1.28 (0.94–1.75)	0.123
	11	124 (16.0)	103 (13.3)	1.04 (0.83–1.30)	0.752
SNP-63	11	720 (82.9)	703 (83.2)	Ref.	
	12	141 (16.2)	130 (15.4)	0.95 (0.73–1.23)	0.695
	22	8 (0.9)	12 (1.4)	0.61 (0.24–1.55)	0.300
SNP-44-43-19-63 haplotypes*	2-1-2-1	32.1	33.9	0.92	0.265
	2-2-2-1	27.1	27.4	0.98	0.876
	1-1-1-1	20.5	19.0	1.10	0.234
	2-1-1-1	11.3	10.8	1.05	0.571
	2-1-1-2	9.0	8.9	1.02	0.942

*OR and *P* values for haplotypes are based on estimated haplotype frequencies. SNP-44 allele 1 = C, allele 2 = T. SNP-43 allele 1 = G, allele 2 = A. SNP-19 allele 1 = 336 bp, allele 2 = 366 bp. SNP-63 allele 1 = C, allele 2 = T. Genotype ORs are calculated versus reference (Ref.). Haplotype frequencies are estimated by the use of Haploview, and frequencies of haplotype combinations are calculated assuming Hardy-Weinberg equilibrium of all markers.

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