Brief Genetics Report

Significant Linkage of BMI to Chromosome 10p in the U.K. Population and Evaluation of *GAD2* as a Positional Candidate

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Obesity is a major health problem, and many family-based studies have suggested that it has a strong genetic basis. We performed a genome-wide quantitative trait linkage scan for loci influencing BMI in 573 pedigrees from the U.K. We identified genome-wide significant linkage (logarithm of odds = 3.74, between D10S208 and D10S196, genome-wide $P = 0.0186$) on chromosome 10p. The size of our study population and the statistical significance of our findings provide substantial contributions to the body of evidence for a locus on chromosome 10p. We examined eight single nucleotide polymorphisms (SNPs) in *GAD2*, which maps to this linkage region, tagging the majority of variation in the gene, and observed marginally significant ($0.01 < P < 0.05$) associations between four common variants and BMI. However, these SNPs did not account for our evidence of linkage to BMI, and they did not replicate (in direction of effect) the previous associations. We therefore conclude that these SNPs are not the etiological variants underlying this locus. We cannot rule out the possibility that other untagged variations in *GAD2* may, in part, be involved, but it is most likely that alternative gene(s) within the broad gene-rich region of linkage on 10p are responsible for variation in body mass and susceptibility to obesity. *Diabetes* 55:1884–1889, 2006

Human obesity is a disease of increasing concern, with obese individuals at increased risk of developing many chronic disorders. By 2004, 49 obesity-related Mendelian syndromes had been localized to genomic regions and 204 quantitative trait loci (QTLs) to obesity-related phenotypes identified from 50 genome scans in humans (1). In addition, nearly 100 candidate genes have been implicated in obesity etiology (1). The close epidemiological and pathological relationship between obesity and type 2 diabetes suggests that their etiology may, in part, be shared. We have analyzed a 573 full sibpair pedigree resource previously ascertained and analyzed for susceptibility to type 2 diabetes (2) for loci influencing BMI.

In our dataset of 573 pedigrees, the phenotypes of age and BMI (both taken at time of ascertainment) were available for nine parents (70–89 years of age) and 1,215 offspring (37–88 years of age) with type 2 diabetes. Mean (±SD) BMI was 27.8 ± 4.3 kg/m^2 in male subjects (n = 657) and 29.8 ± 5.6 kg/m^2 in female subjects (n = 567). Measures of BMI were log transformed to reduced skewness and kurtosis. To account for possible effects of age, sex, and selection (due to ascertainment on the basis of type 2 diabetes status), we adjusted and standardized our sample data against U.K. population data obtained through the Health Survey for England 1998 (3). Linkage analysis was performed using the combined "squared sums-squared differences" Haseman-Elston regression approach, implemented in MERLIN-REGRESS (4). This method properly accommodates the selected nature of our data by preventing inflation of the type I error rate and achieving maximal power during linkage analysis; therefore, it has advantages in this situation over variance components–based methods (4). We estimated sibling genotype identity-by-descent coefficients using genotypes generated from 418 autosomal microsatellite markers [mean spacing 9.26 cM(H)] (2). We specified the sex- and age-adjusted population mean (zero), variance (one), and heritability (60%) (5) in our analyses. Power calculations indicate a power of ~70% to detect linkage to a locus accounting for 30% of the total phenotypic variance with a logarithm of odds (LOD) score of 2 and ~45% power to detect a linkage with an LOD score of 3 (6).

Our genome-wide scan for BMI susceptibility genes identified two linkage peaks with an LOD ≥1.18: one on chromosome 1p36.13 with an LOD score of 1.38 between markers D1S2697 and D1S199 and a second, much larger peak on chromosome 10p11.22-23 with an LOD of 3.74 between markers D10S208 and D10S196 (Fig. 1). We determined by simulation that the signal on chromosome 10 was significant at the genome-wide level ($P = 0.0186$) and the point-wise level ($P = 0.0001$) using an approach that has been previously described (6). Our original genome-wide linkage analysis of type 2 diabetes susceptibility in this dataset (2) identified only marginally significant

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Received for publication 23 December 2005 and accepted in revised form 3 March 2006.

LD, linkage disequilibrium; LOD, logarithm of odds; QTDT, quantitative transmission-disequilibrium test; QTL, quantitative trait locus; SNP, single nucleotide polymorphism.

DOI: 10.2337/db05-1674

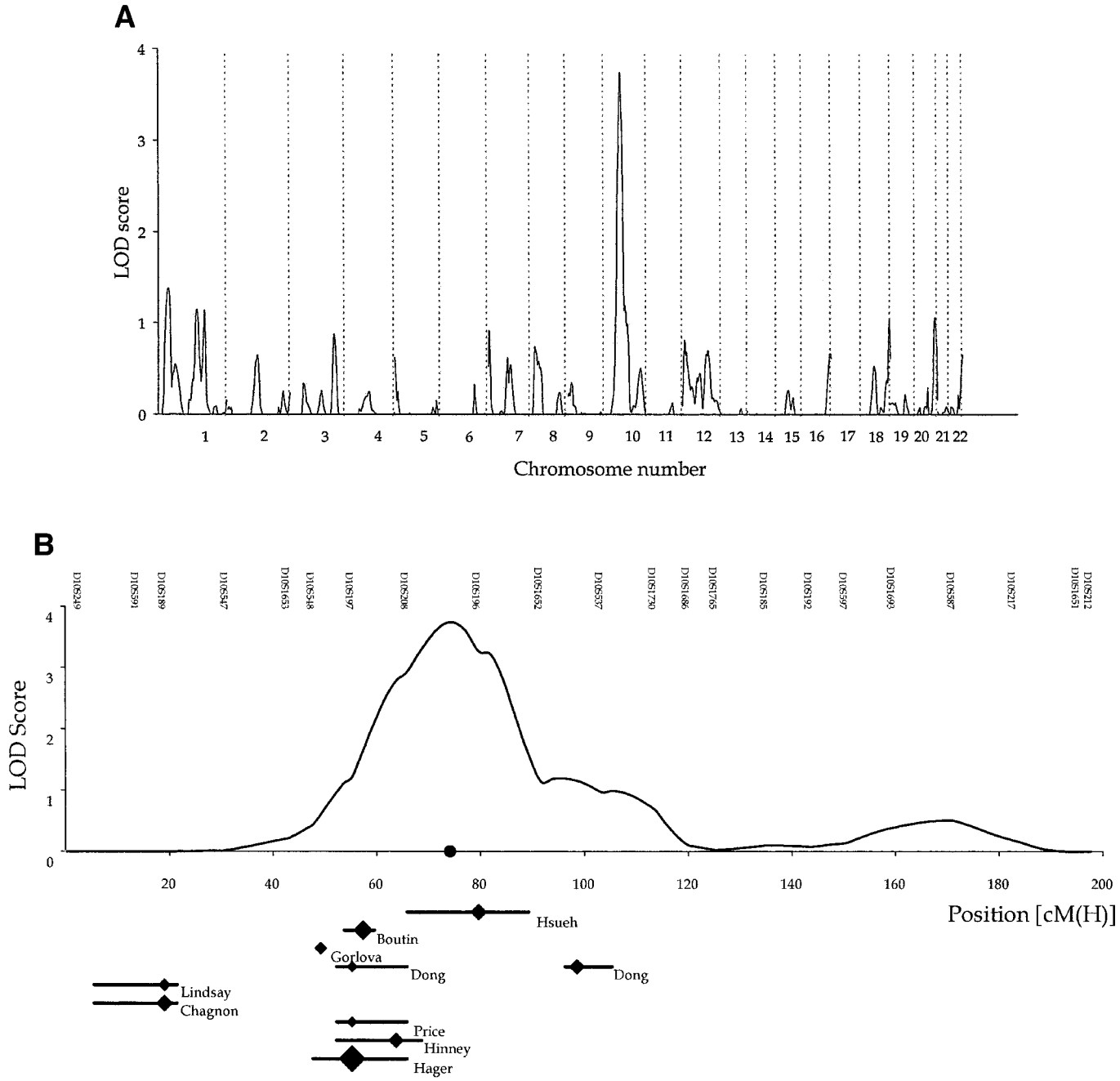


FIG. 1. *A*: A genome-wide quantitative trait multipoint linkage analysis of BMI in 573 pedigrees. The vertical broken lines indicate chromosome boundaries. The analysis was carried out using MERLIN-REGRESS, as described in the text. *B*: Chromosome 10 quantitative trait multipoint linkage analysis of BMI. The microsatellite markers used in the analysis are shown along the *top*. The position of the centromere is indicated on the *x*-axis. The linkage results from nine previous studies of BMI and obesity are shown below the *x*-axis by diamonds. The sizes of diamonds are proportional to −log(*P* value) of the maximum LOD (or nonparametric linkage) scores reported in the respective articles, the positions of which represent the locations of the linkage peaks, converted where appropriate into Haldane centimorgan units [cM(H)], from the respective reports. Locus boundaries (indicated by horizontal lines) were either undefined (15), author defined (7,11,14), or estimated as a 1-LOD support interval (8–10,12,13).

linkage to this region on 10p, with an LOD of 0.68 (asymptotic point-wise $P = 0.039$). Conversely, we observed no linkage of BMI (with asymptotic point-wise $P < 0.05$) to the region on 10q23 in which we had previously observed maximal evidence for linkage to type 2 diabetes. We can therefore be confident that the linkage evidence on 10p reflects a locus influencing BMI susceptibility directly rather than the surrogate effects of a type 2 diabetes susceptibility locus.

Our study adds substantially, in terms of significance of results and sample size, to the growing body of evidence for linkage to this region. Nine previous studies have reported evidence for linkage to BMI or related traits on the short arm or pericentric region of chromosome 10 (7–15). The majority of these studies, our own included, demonstrate maximum evidence for linkage in the region 55–80 cM from the p-terminal of chromosome 10 (Fig. 1*B*), strongly implicating a locus in this region that influences susceptibility to body mass and obesity.

Several excellent candidate genes fall within this region

TABLE 1
Tests of total association of individual *GAD2* SNPs with BMI

GAD2 SNP(s)	Total association (*P* value)		Trait means (*Z* scores)*	
	QTDT	QPDTPHASE	Common allele	Minor allele
Initial three SNPs				
rs2236418	0.025	0.051	0.28	0.13
rs992990	0.092	0.055	0.29	0.19
rs928197	0.011	0.017	0.29	0.12
Subsequent five SNPs				
rs2839669	0.036	0.038	0.29	0.15
rs7071922	0.031	0.029	0.30	0.17
rs3781117	>0.1	0.996	0.27	0.25
rs7908975	>0.1	0.555	0.27	0.25
rs3781107	>0.1	0.432	0.28	0.22

*Mean trait values from QPDTPHASE. The trait values are *Z* scores (i.e., log-transformed BMI adjusted for the effects of age and sex and standardized against the U.K. population [3]).

on chromosome 10, including *GAD2*, *UCN3* (urocortin III), and *PPYR1/NPY4R* (pancreatic polypeptide receptor 1). *GAD2* encodes GAD65, which catalyzes the formation of the neurotransmitter γ aminobutyric acid (GABA), is synthesized by GABAergic neurones within nuclei of the hypothalamus that are known to be involved in feeding behavior (16), and has been the focus of three recent studies.

The fine-mapping linkage study of adult obesity in the French population by Boutin et al. (13) observed that the maximally linked microsatellite marker in their study, D10S197, was located within intron 7 of *GAD2*. Boutin et al. identified three single nucleotide polymorphisms (SNPs), a promoter variant (rs2236418, −243 A>G), and two intronic variants (rs992990, +61450 C>A and rs928197, +83897 T>A) that are associated with obesity. In each case, the rare allele was associated with an obese phenotype. They observed marginally significant association between the variant allele of rs992990 and evidence for linkage ($P = 0.02$), together with functional evidence implicating the other two SNPs. The second study, also in a French population, observed that the same (G) allele of the promoter SNP (rs2236418) was associated with severe childhood obesity (17). Most recently, Swarbrick et al. (18) investigated the importance of *GAD2* in obesity susceptibility in two large case/control studies (in American and Canadian populations) and in a smaller study of German pedigrees and found no statistical evidence supporting the candidacy of *GAD2*.

Given this prior, albeit conflicting, evidence of the importance of *GAD2* in body mass determination, we examined the candidacy of *GAD2* in our population. We first considered the three SNPs (rs2236418, rs992990, and rs928197) that showed some evidence of association in the previous studies. The 573 sibships were genotyped for these three SNPs using a competitive allele-specific PCR method (KASPar; Kbioscience, Hertfordshire, U.K.). Genotyping call rates exceeded 95% at each SNP, and no discordant genotypes were observed in 92 duplicate samples.

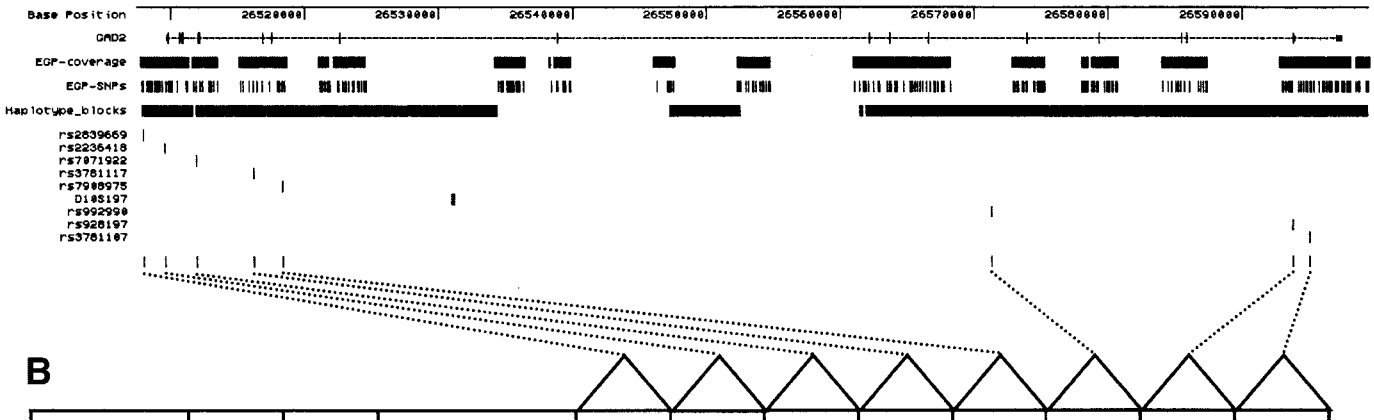
We found no evidence for population stratification with the three SNPs analyzed (all $P \geq 0.5$). This fits with previous studies of these case samples when STRUCTURE has been applied to the genome-wide microsatellite data (N. Martin, L. Cardon, personal communication) and a more recent genome-wide analysis using the quantitative transmission-disequilibrium test (QTDT) (19,20) (all $P \geq 0.6$, corrected for multiple testing). We therefore determined the total evidence for association of each SNP with adjusted and standardized BMI using QTDT, as well as with QPDTPHASE (21), which makes less restrictive assumptions about the data distribution (22). We observed associations of marginal significance with two of these SNPs, rs2236418 ($P = 0.025$) and rs928197 ($P = 0.011$) (Table 1). However, in both cases, the minor allele was associated with lower mean BMI, in contrast to the findings of Boutin et al. To capture additional genetic diversity within *GAD2*, we used HAPLOVIEW (23) and the *GAD2* resequencing data from the Environmental Genome Project (www.egp.gs.washington.edu) to select an additional five SNPs for genotyping. The resulting eight-SNP panel tagged all of the haplotypes in the three largest linkage disequilibrium (LD) blocks (blocks 1, 2, and 5, spanning 73% of the total gene sequence) (Fig. 1*A*). The gene also contained two small blocks (blocks 3 and 4) and the intervening regions of low LD that would have required substantial additional genotyping to tag. These eight SNPs tagged the proximal promoter, the 3′ untranslated region, and all exons but one, capturing 93% of all common

TABLE 2
Tests of association of block-tagging *GAD2* SNP combinations with BMI

Block tagging *GAD2* SNP combination	Global QPDTPHASE (*P* value)*	Significant haplotypes {frequency} (trait mean) [*P* value]†	
rs2839669, rs2236418	0.049	11 {0.901} (0.29) [0.051]	22 {0.097} (0.14) [0.038]
rs7071922, rs3781117, rs7908975	0.044	111 {0.639} (0.31) [0.048]	211 {0.166} (0.14) [0.010]
rs992990, rs928197, rs3781107	0.032	111 {0.629} (0.31) [0.025]	221 {0.119} (0.13) [0.014]

*Haplotypes of these SNPs are examined in a global (or omnibus) test, with rare (<1% frequency) haplotypes pooled. †Significant haplotypes from the haplotype-specific comparisons from QPDTPHASE. 1, common allele; 2, rare allele. For all three *GAD2* SNP combinations, only two haplotypes were significant in the haplotype-specific comparisons. The frequency and mean trait values (*Z* scores) for each haplotype are shown. *P* values (all uncorrected for multiple testing) are for the haplotype in question tested against all others pooled.

A



B

SNP	MAF	LD block	Position (NCBI Build 35)	rs2839669	rs2236418	rs7071922	rs3781117	rs7908975	rs992990	rs928197	rs3781107
rs2839669	0.168	1	26507808		0.993	0.562	0.032	0.012	0.455	0.826	0.029
rs2236418	0.161	1	26545502	1.000		0.573	0.032	0.011	0.459	0.807	0.029
rs7071922	0.254	2	26547853	1.000	1.000		0.056	0.300	0.796	0.462	0.045
rs3781117	0.141	2	26552050	0.996	0.997	0.998		0.016	0.023	0.034	0.813
rs7908975	0.091	2	26554218	0.782	0.772	1.000	0.980		0.238	0.015	0.008
rs992990	0.275	5	26607187	0.947	0.947	0.948	0.601	0.948		0.529	0.051
rs928197	0.172	5	26629631	0.931	0.924	0.877	0.998	0.846	0.992		0.031
rs3781107	0.130	5	26630940	0.995	0.995	0.939	0.996	0.716	0.944	0.996	

FIG. 2. Genomic structure and LD relationships of the *GAD2* gene. *A*: *Top to bottom*: Base pair position (National Center for Biotechnology Information build 35), genomic structure of *GAD2*, sequence coverage of the gene by Environmental Genome Project (EGP), SNPs discovered by EGP, and haplotype block structure based on EGP SNP data. The eight SNPs in the present study are shown in full and condensed. *B*: Genomic positions, minor allele frequencies, and pairwise LD relationships (r^2 measures above the diagonal and D′ measures below) for the eight SNPs typed in the present study.

(>10%) variation with an $r^2 > 0.5$ and 35% of that with an $r^2 > 0.8$ across the gene as a whole. The arrangement of the SNPs, the LD blocks that they tag, and their r^2 and D′ values are shown in Fig. 2. We tested the five additional SNPs individually for total association with BMI using QTDT and QPDTPHASE, having first confirmed no evidence for population stratification at these markers (all $P \geq 0.6$). We also tested haplotypes of block-tagging SNPs (Table 2) with QPDTPHASE, pooling haplotypes with frequencies <1%. We found SNP rs2839669 ($P = 0.036$), located upstream of the associated promoter SNP rs2236418, and rs7071922 ($P = 0.031$), located in block 2, to be marginally associated, with the minor allele again associated with lower BMI (Table 1). Haplotypes of the block-tagging SNPs were also marginally associated with BMI (Table 2). No global association was seen with microsatellite D10S197 (genotyped in the original genome scan), located proximal to rs7908975 in block 2 ($P > 0.1$). No SNP showed evidence of dominance ($P > 0.1$).

We determined the extent to which these associated SNPs accounted for the linkage signal. The evidence for linkage to a quantitative trait is reduced when an association with an SNP is incorporated into the test for linkage. When the associated SNP is in perfect LD with the QTL, or is the QTL itself, the evidence for linkage can be eliminated completely (24). We modeled each associated SNP as a covariate, with genotype "11" coded as −1, "12" as 0, and "22" as +1, consistent with a standard biometrical model and the gene-dosing approach used by QTDT (20). We also modeled the three sets of block-tagging SNPs as a set of covariates representing each individual SNP. We regressed each individual's *GAD2* covariate(s) from BMI using coefficients calculated with QTDT. To allow a meaningful interpretation of these *GAD2* covariate analyses, we obtained the appropriate baseline multipoint LOD scores for each SNP in question, that is, the evidence for linkage alone, without accounting for the association, restricted to those family members with a successful *GAD2* genotype and covariate.

For these analyses, we used the latest version of MERLIN-REGRESS, which accommodates the effects of LD between SNPs (known to inflate linkage evidence if not properly accommodated) by grouping them into clusters for the purposes of calculating allele-sharing probabilities (25). However, this method assumes no recombination within clusters and no LD between clusters. To accommo-

TABLE 3
Tests of multipoint linkage and association of *GAD2* with BMI

Associated *GAD2* SNP(s)	Analyses assuming one SNP cluster		Analyses assuming three SNP clusters	
	Linkage alone (LOD)	Linkage given association (LOD)	Linkage alone (LOD)	Linkage given association (LOD)
rs2839669	3.88	3.54	4.32	3.94
rs2236418	4.08	3.58	4.62	4.09
rs2839669, rs2236418	4.19	3.79	4.77	4.31
rs7071922	2.88	2.79	3.25	3.12
rs7071922, rs3781117, rs7908975	2.80	2.55	3.18	3.00
rs928197	3.63	3.14	4.19	3.63
rs992990, rs928197, rs3781107	3.28	2.88	3.84	3.47

Linkage alone: baseline multipoint LOD score for linkage to BMI calculated only in those individuals who have genotypes for the *GAD2* SNP in question. Linkage given association: multipoint LOD score for linkage to BMI after the *GAD2* association has been accounted for, by adjustment on the *GAD2* covariate.

date the high multiallelic D′ (i.e., nonzero LD) between the haplotype blocks in *GAD2*, we used two alternative clustering approaches. In the first, we treated the eight SNPs as three separate clusters (representing the three blocks they were chosen to tag), with the intervening microsatellite (D10S197, falling within block 2) considered as part of the second cluster. In the second, we grouped all SNPs (together with D10S197) into one single cluster.

We found that the baseline LOD scores differed according to the clustering approach used to model LD (Table 3, *columns 2* and *4*). However, the effects of the *GAD2* covariates themselves did not differ appreciably between clustering methods (Table 3, *columns 3* and *5*). All four associated SNPs, and the haplotypes containing them, resulted in very minor LOD score reductions ($\Delta LOD < 0.6$; Table 3). These results suggest that not one of the SNPs tested is the QTL responsible for the linkage to BMI.

In conclusion, we have detected genome-wide significant linkage to BMI on the pericentric region of chromosome 10 in U.K. pedigrees with type 2 diabetes. This is the largest dataset to observe linkage to this region, so our study substantially adds to the body of evidence implicating loci in this region in BMI variation. We have examined the candidacy of *GAD2* as the gene underlying this linkage, following two previous studies reporting association. We observed marginally significant associations to four SNPs in *GAD2*. Of the three SNPs in common with the Boutin study, we only observed associations with rs2236418 ($P = 0.025$) and rs928197 ($P = 0.011$), but in the opposite direction. Differences in the underlying LD structure of chromosome 10 in U.K. and French populations are highly unlikely explanations for this reversal of association. It is, in principle at least, conceivable that differences in genetic background, patterns of interacting environmental exposures, and clinical features between the two samples could explain a reversal of association direction. However, such differences would have to be extreme, which seems implausible given that these are otherwise quite similar European samples. We note that the Boutin study examined extreme obesity in a case/control setting, whereas we have quantitatively examined a continuous distribution of BMI measurements, only a very small proportion of which could be described as extremely obese. While such differences in study design might in theory explain a failure to reproduce the finding of an effect, it is difficult to see how these differences could explain a change in the direction of an effect. We conclude that our findings do not constitute replication (defined as same allele/haplotype, same phenotype) of the associations seen in previous studies.

Given the number of SNPs that we have tested, and the low correlation between them, our association findings may reflect chance. The alternative is that the associations we observe are true and reflect modest LD with other etiological variants. While it is possible that these variants lie in *GAD2*, as we have not exhaustively surveyed all genetic variation therein, it is more likely that they are located elsewhere in the ~30-Mb region encompassing the consensus of linkage peaks.

ACKNOWLEDGMENTS

The Warren 2 family collection was funded by Diabetes UK through the Warren 2 Bequest and the genotyping through project grant RD02/0002385. S.W. is funded by a Wellcome Trust Career Development Fellowship.

We thank Drs. Stacey Cherny and Javier Gayan (Wellcome Trust Centre for Human Genetics) for useful discussions. Prof. Paola Primatesta (University College London) kindly made available population data from the Health Survey for England.

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