Original Article

Variation Within the Gene Encoding the Upstream Stimulatory Factor 1 Does Not Influence Susceptibility to Type 2 Diabetes in Samples From Populations With Replicated Evidence of Linkage to Chromosome 1q

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The gene encoding the transcription factor upstream stimulatory factor (USF)1 influences susceptibility to familial combined hyperlipidemia (FCHL) and triglyceride levels. Phenotypic overlap between FCHL and type 2 diabetes makes USF1 a compelling positional candidate for the widely replicated type 2 diabetes linkage signal on chromosome 1q. We typed 22 variants in the *F11R/USF1* region (1 per 3 kb), including those previously implicated in FCHL-susceptibility (or proxies thereof) in 3,726 samples preferentially enriched for 1q linkage. We also examined glucose- and lipid-related continuous traits in an overlapping set of 1,215 subjects of European descent. There was no convincing evidence for association with type 2 diabetes in any of seven case-control comparisons, individually or combined. Family-based association analyses in 832 Pima subjects were similarly negative. At rs3737787 (the variant most strongly associated with FCHL), the combined odds ratio, per copy of the rarer A-allele, was 1.10 (95% CI 0.97–1.24, $P = 0.13$). In 124 Utah subjects, rs3737787 was significantly associated ($P = 0.002$) with triglyceride levels, but direction of this association was opposite to previous reports, and there was no corroboration in three other samples. These data exclude *USF1* as a major contributor to type 2 diabetes susceptibility and the basis for the chromosome 1q linkage. They reveal only limited evidence for replication of *USF1* effects on continuous metabolic traits. *Diabetes* 55:2541–2548, 2006

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DI, disposition index; FCHL, familial combined hyperlipidemia; FSIGT, frequently sampled intravenous glucose tolerance test; LD, linkage disequilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism; USF, upstream stimulatory factor.

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Positional cloning within regions previously highlighted by genome-wide linkage analysis represents one of the dominant strategies for identification of sequence variants influencing individual risk of type 2 diabetes. A region of chromosome 1q (from 147 to 175 Mb on the NCBI35 assembly) has emerged as among the strongest signals (1) with linkage to type 2 diabetes demonstrated in diverse populations of European (2–7), East-Asian (8,9), Native-American (10), and African-American origin (S.C.E., unpublished observations). Moreover, signals for several diabetes-related traits have also been mapped to this region (11–14). The International Type 2 Diabetes 1q Consortium represents a collaborative effort between many of the groups with 1q linkage signals to identify the variant(s) responsible. In addition to dense-map indirect linkage disequilibrium (LD) mapping of the region, the most compelling positional candidates are being targeted for detailed analysis.

The gene encoding upstream stimulatory factor (USF)1 is one such candidate. *USF1* maps in the middle of the region of interest (~158 Mb), close to the peak of linkage in several of the type 2 diabetes-linked populations

TABLE 1
Characteristics of the samples studied

	n	Male (%)	Ethnicity	Age at diagnosis or age at study (years)*	BMI (kg/m^2)
U.K.					
Sibpair probands	449	54	European descent	54.7 ± 8.1	28.8 ± 5.1
Control subjects	450	56	European descent	—	—
French					
Diabetic case subjects	259	47	European descent	43.2 ± 9.5	26.2 ± 3.8
Control subjects	288	37	European descent	59.9 ± 13.6*	24.9 ± 4.2
Shanghai					
Sibpair probands	80	50	East Asian	35.3 ± 3.9	23.1 ± 3.4
Chinese control subjects	80	69	East Asian	74.2 ± 5.5*	21.1 ± 2.3
Hong Kong					
Sibpair probands	64	42	East Asian	38.0 ± 8.7	27.8 ± 4.0
Control subjects	64	42	East Asian	42.2 ± 8.9*	21.5 ± 2.0
Utah					
Case subjects	182	70	European descent	62.7 ± 9.8	31.5 ± 5.8
Control subjects	167	39	European descent	51.3 ± 14.9*	28.1 ± 6.3
Intermediate trait	124	43	European descent	40.0 ± 11.1*	27.9 ± 6.0
Amish					
Familial case subjects	150	32	European descent	59.6 ± 11.2	29.4 ± 5.7
Control subjects	361	52	European descent	51.7 ± 10.5*	27.4 ± 4.6
Other samples	203	38	European descent	50.5 ± 16.7*	28.3 ± 5.3
Pima					
Early-onset case subjects	200	35	Native American	17.8 ± 4.5	35.1 ± 8.1
Elderly control subjects	199	50	Native American	61.3 ± 9.3*	32.1 ± 6.3
Other family members	733	37	Native American	36.3 ± 10.4 49.6 ± 6.9*	33.6 ± 8.1
Arkansas					
Intermediate trait	181	38	European descent	35.7 ± 9.1*	29.7 ± 6.0

Data as means ± SD. Age at diagnosis was taken for case subjects only. *Age at study was taken for control subjects or other nondiabetic subjects.

(2,3,8,10). The gene encodes a ubiquitously expressed member of the basic helix-loop-helix leucine zipper transcription family, which, either as a homodimer or through heterodimerization with the related USF2 protein, binds the E-box motif found in the promoters of many genes involved in glucose and lipid metabolism (15,16). Although the relative functional importance of USF1 and USF2 (and of sterol regulatory element–binding protein 1, which interacts with the same motif) remains unclear (17,18); considerable evidence implicates the USFs in the regulation of key metabolic genes including liver pyruvate kinase (19), fatty acid synthase (16,20), glucokinase (21), and pyruvate dehydrogenase complex component X (22).

The biological plausibility of *USF1* as a positional candidate for type 2 diabetes is enhanced by evidence implicating the gene in susceptibility to familial combined hyperlipidemia (FCHL) (23,24). In Finnish pedigrees segregating FCHL, in which prior evidence for 1q linkage had been reported (25), several single nucleotide polymorphisms (SNPs) within *USF1* (and to a lesser extent, within the neighboring *F11R* [or *JAM*] gene) were associated with FCHL and triglyceride levels. Though no single causative variant was identified, the strongest associations involved the common allele at two highly correlated SNPs, rs3737787 (within the 3′ untranslated region) and rs2073658 (within intron 7). These findings were recently replicated in families from Utah (24). Given the strong phenotypic overlap between FCHL and type 2 diabetes (which is characterized by an FCHL-like dyslipidemia) and growing suggestions that the β-cell dysfunction and insulin resistance of type 2 diabetes may, in fact, be secondary to disturbances of lipid metabolism, *USF1* emerges as a strong candidate vis-à-vis the 1q linkage signal.

There have been only limited studies addressing the role of *USF1* variation and glucose metabolism to date. In 800 young men from the European Atherosclerosis Research Study, Putt et al. (26) tested three intronic *USF1* SNPs (one of which, rs2073655, was in complete LD with the SNPs displaying the strongest FCHL association signal in Finns) reporting weak haplotypic associations with glucose levels during an oral glucose tolerance test. A recent study in Hong Kong Chinese reported associations of *USF1* SNPs (including rs3737787) with type 2 diabetes and metabolic syndrome in one case-control analysis (using family cases showing 1q linkage), but these were not replicated in a second Chinese sample (27). Most recently, a study of eight *USF1* SNPs (including rs3737787 and rs2073658) reported no association with type 2 diabetes in 1,475 French case-control samples (28).

The present study has made use of dense-map, large-scale genotyping data from the consortium's mapping efforts on chromosome 1q to evaluate the influence of *USF1* variation on type 2 diabetes risk and related metabolic traits, making explicit use of populations and samples selected because of their prior evidence for linkage to the region.

RESEARCH DESIGN AND METHODS

We used three complementary approaches to the analysis of *USF1* variation: *1*) a large multiethnic case-control analysis in seven 1q-linked populations, *2*) family-based association analyses in an enlarged Pima dataset, and *3*) analysis

of diabetes-related quantitative traits in Amish, Utah, and Arkansas subjects of European origin.

Multiethnic case-control study. We examined 2,993 case-control samples from seven populations with evidence for 1q linkage (Table 1 and online appendix [available at http://diabetes.diabetesjournals.org]). For five of the populations, cases included probands from some (U.K.) or all (French, Shanghai, Hong Kong, and Utah) pedigrees from the relevant genome scans (2–5,8,9), allowing us to exploit the increase in power when cases are selected for membership of a multiplex sibship (29). In French and Utah samples, additional cases were recruited from other sources (online appendix). In these five samples, cases were unrelated, and ethnically matched locally recruited control subjects were used (online appendix).

All of the Amish and a quarter of the Pima case-control samples were recruited from the pedigrees typed in the relevant linkage studies (7,10). The Pima case-control sample compared 200 case (age of diagnosis <25 years) with 199 control subjects (nondiabetic after age 45 years). No case or control subject was a first-degree relative of another individual in the sample. The Amish case-control set consisted of 150 type 2 diabetic case and 361 control subjects (normal glucose tolerance beyond the age of 38 years). Given sustained endogamy within Amish communities, ascertainment of unrelated case and control subjects was not feasible. Thus, analyses in the Amish take account of the correlations among related individuals (see below).

Differences in clinical parameters of case and control subjects (Table 1) are consistent with recognized ethnic and geographic variation in anthropometry and the clinical presentation of type 2 diabetes (for example, the early onset of diabetes in Pima Indians and the relatively low adiposity of Chinese subjects). Note that the Hong Kong and French samples reported here represent a subset of those separately analyzed for *USF1* variation at selected SNPs by Ng et al. (27) (64 case and 64 control subjects in common) and Gibson et al. (28) (172 case and 282 control subjects in common).

Family-based association study. A further 733 Pima samples from the original linkage pedigrees were typed and, with 99 individuals from the case-control sample, analyzed using family-based association methods. These 832 Pima samples include 570 type 2 diabetic subjects (diagnosed before age 45 years), 104 nondiabetic siblings (aged >45 years), and 158 parents (to reconstruct family relationships).

Quantitative trait study: Amish. In addition to case-control samples, the 1q consortium genotyped a further 203 Amish individuals from the same pedigrees, including 159 with impaired glucose tolerance and/or impaired fasting glucose and 44 "linking" individuals to allow pedigree reconstruction. Fasting levels of (serum) total and HDL cholesterol and triglycerides (Quest Diagnostics, Baltimore, MD) were available for 631 of 714 individuals, including 103 type 2 diabetic case subjects, 342 subjects with normal glucose tolerance, 150 subjects with impaired glucose homeostasis, and 36 of the linking subjects (24 with normal and 12 with unknown glucose tolerance).

Utah and Arkansas. To examine the impact of *USF1* SNPs on insulin sensitivity (S_i), insulin secretion (AIR_g), and β-cell function (disposition index [DI]), we analyzed two samples. The first consisted of 124 nondiabetic members of Utah families with evidence of chromosome 1q linkage (2,5). The second sample included 181 unrelated Arkansas Caucasians with normal glucose tolerance tests, ascertained primarily for age 18–50 years. Characteristics of the latter population, not previously reported, are summarized in Table 1.

All Utah subjects and 105 of those from Arkansas underwent a tolbutamide-modified frequently sampled intravenous glucose tolerance test (FSIGT) (30,31). Subjects on lipid-lowering medications were excluded from analysis. Tolbutamide became unavailable during the Arkansas study, and the remaining 76 were studied with an insulin-modified FSIGT. After baseline sampling, the FSIGT was initiated with a 11.4 g/m^2 50% dextrose bolus over 1 min; a further 25 samples were collected over 3 h. At 20 min, subjects were given tolbutamide (125 mg/m^2) or insulin (0.04 units/kg). If the glucose between 120 and 180 min differed by >0.25 mmol/l or showed a clear upward or downward trend, sampling was continued to 240 min.

In addition, we analyzed trait data from those members of the Utah case-control population (see above) with BMI, cholesterol, and triglyceride measures (116 case and 163 control subjects). Subjects from this group on lipid-lowering medications were not excluded from analyses.

For Utah samples, insulin was measured with a radioimmunoassay with appreciable proinsulin crossreactivity. For Arkansas samples, insulin was measured by the General Clinical Research Center Core Laboratory using an insulin-specific immunochemilluminometric assay (MLT Assay, Wales, U.K.). Plasma glucose was measured by a glucose oxidase assay, and blood lipids were measured using standard clinical assays by LabCorp (Burlington, NC).

Ethical information. All samples were ascertained with written informed consent under protocols approved by respective local ethics committees or institutional review boards. Use of samples for 1q consortium purposes was, where necessary, supplemented by further specific approval. The Oxfordshire Local Research Ethics Committee assessed all ethical documentation and provided specific approval for each sample genotyped in the U.K.

SNPs and genotyping. 1q consortium samples were typed for 22 SNPs covering *USF1* and *F11R* (Fig. 1). These included 15 SNPs typed as part of the consortium's effort to cover the 1q region of interest at 5 kb density and 7 SNPs specifically chosen given the FCHL association data (23). All SNPs were typed using the Illumina Golden Gate assay (32) at the Wellcome Trust Sanger Institute, as part of two 1536-plex assays.

The Utah and Arkansas intermediate trait samples were independently genotyped at the University of Arkansas using Pyrosequencing (PSQ-96; Biotage, Uppsala, Sweden). Using genotypes gathered in 96 Utah subjects for 10 *USF1* SNPs selected from prior data (23), six tag SNPs (rs1556259, rs2516838, rs2516839, rs2516840, rs2516841, and rs3737787) were defined using pairwise LD methods (LDSelect; $r^2 > 0.9$) (33). Further details of primer sequences and assay conditions are available from the authors.

Data quality control. Consortium genotyping data from the Illumina platform were subjected to extensive quality control, which required SNPs to pass criteria for call rates, Illumina quality score, and duplicate error rates. We also tested for departures from Hardy-Weinberg equilibrium and expected allele frequencies, for excessive within-sample plate-to-plate variation, and, in Pima and Amish samples, for Mendelian inconsistency. As a result, SNPs rs3813609 and 7556492 were excluded from analysis in all populations; rs790055 from Pima, French, Shanghai, and U.K. populations; rs2988726 from French, Shanghai, and U.K. populations; and rs2774279 from the Hong Kong population. For SNPs passing this quality control pipeline, we estimate, on the basis of ~450,000 cryptic duplicate genotypes, an overall genotyping error rate of ~0.2%.

Statistical methods

Multiethnic case-control study. For the case-control samples, between-group differences in genotype frequency were evaluated on a population-specific basis using standard contingency table methods with the additive model as default. Exact *P* values were calculated, where necessary, using Stata SE version 8 and STATXACT (Cytel, Cambridge, MA). In the Amish sample, population structure results in substantial relatedness within (and between) case and control subjects; failure to allow for this will underestimate the variance and upwardly bias the test statistic. We therefore repeated case-control comparisons in the Amish by modeling a residual familial effect as a random effect using SOLAR. Specifically, we used the measured genotype approach to assess the effect of genotype on diabetes status while simultaneously estimating the residual correlations in phenotype among related individuals (34).

Single-point data from the seven case-control samples were combined using the Mantel-Haenszel fixed-effects method (Stata SE version 8 and STATXACT). For these analyses, we first established the homogeneity of odds ratios (ORs) across combined strata before generating combined ORs under dominant, additive, and recessive models. The Mantel-Haenszel method assumes independence of case and control data. To account for departures from this assumption in the Amish, this analysis was repeated using an inverse variance-based method (35). The logarithm of the OR (logOR) (under the additive model) and its variance were calculated for each population by logistic regression and the study-wide common logOR estimated as the weighted sum of the study-specific values, with weights taken as the inverse of the variance estimates. In the Amish, the corrected value of the logOR variance was taken as the *P* value for the measured genotype approach using the Wald statistic. Pairwise measures of LD were calculated, and haplotype analyses were performed using haplotype trend regression (36) implemented in HelixTree (GoldenHelix, Bozeman, MT).

Family-based association analysis. Analysis of the extended Pima data set ($n = 832$) was conducted in two ways. First, case-control comparisons were made across the full resource (using a cutoff for age at diagnosis of 45 years) using binomial generalized estimating equations to control for family membership. Second, we conducted an explicit family-based association analysis using the method of Abecasis (37) to partition the association into between- and within-family components.

Quantitative trait analyses. In the Amish, full pedigree structure was explicitly specified and analyses conducted in a regression framework with trait measures as the dependent variable. In Utah and Arkansas datasets, S_i was estimated from glucose and insulin measures during the FSIGT using the MinMod (Utah) or MinMod Millennium (Arkansas) programs (38,39). AIR_g was calculated as the mean insulin response above basal from 2 to 10 min following the glucose bolus and DI as $S_i \times AIR_g$. Genotypic effects on S_i, AIR_g, DI, and lipids were tested using mixed-effect general linear models in SPSS version 12. Primary tests were conducted under an additive model unless homozygosity for the rare allele was observed in <10 cases, in which case a recessive model for the common allele was tested. Skewed variables (S_i, AIR_g, DI, BMI, triglycerides, and free fatty acid levels) were ln-transformed to normality before analysis. All models included age (and BMI, as appropriate)

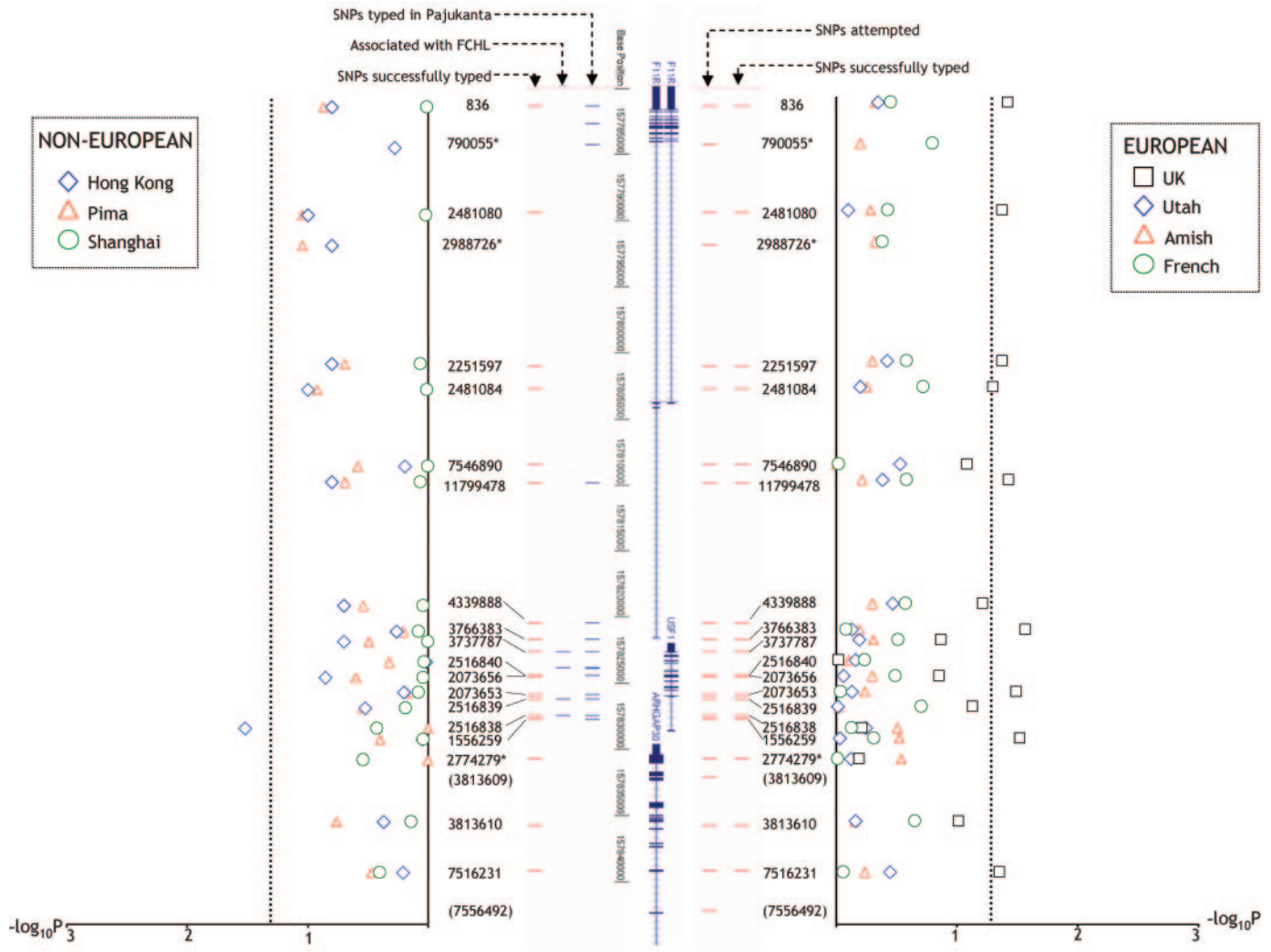

FIG. 1. Single-point case-control association analyses for the *USF1* gene region. The central part of the figure shows the regional gene structure (for *USF1*, *F11R*, and *ARHGAP30*), the SNPs typed, and those associated with FCHL or triglycerides (Pajukanta et al. [23]), as well as the SNPs attempted in this study ($n = 22$) and those successfully typed in at least one population ($n = 20$). SNPs rs7556492 and 3813609 are shown in parentheses, as they failed quality control in all populations. The three asterisked SNPs failed only in some populations. The *right panel* displays the case-control association *P* values for the four samples from European descent. For the Amish, these are corrected for family structure by the measured genotype method. The *left panel* displays the case-control association *P* values for the East-Asian and Pima samples. The dotted vertical lines define $P = 0.05$ ($-\log_{10}P = 1.3$).

as covariates and sex and genotype as fixed factors. Additionally, diagnosis and pedigree membership were included in analysis of family members from the Utah study and protocol type (tolbutamide or insulin) as a fixed factor for Arkansas subjects. The significance of associations detected was examined by comparison of marginal means using the least significant difference test. We considered $P < 0.05$ to be significant throughout without correction for multiple testing.

RESULTS

Multiethnic case-control study. Following quality control, between 18 and 20 (depending on the population) of the original 22 SNPs (covering a 60-kb range) were available for analysis. Within the immediate *USF1* region (~11 kb), complete data were available from nine SNPs. These include three of four SNPs showing association in the Finnish FCHL study (23) (rs3737787, rs2516839, and rs2516838 but not rs2073658 for which assay design failed). Using deep resequencing and genotype data from Seattle SNPs (http://pga.mbt.washington.edu/), these typed SNPs discriminate over 93% of all *USF1* haplotypes observed in 46 chromosomes of European origin (SNPs with minor allele frequency [MAF] >5%) and tag >95% of all SNPs with MAF >5% (the exception being rs10908821 and its associated haplotype, both with a frequency of 7%) with a $r^2 > 0.8$. The untyped FCHL-associated SNP (rs2073658) is in almost complete LD with rs3737787 in samples of European descent (23,26). LD plots for the seven samples are shown in online appendix Table 1.

Single-point results for each of the seven case-control populations are summarized in Fig. 1 and detailed in online appendix Table 2. The Amish case-control data shown were derived using the measured genotype approach to allow for family structure (34). There was no suggestion of association with rs3737787 in any population. In the U.K. case-control analysis, nominally significant ($P < 0.05$) associations were seen for several variants, including a pool of highly correlated SNPs within *F11R* and rs2073653 and rs1556259 within *USF1* but none was replicated in the other samples (see Fig. 1). In the Hong Kong sample, there was nominal evidence of an association with type 2 diabetes at rs2516838 ($P = 0.03$, additive model); again, this was not substantiated in other samples. Finally, haplotype analyses of the nine core USF1

TABLE 2
Single SNP analyses for association with type 2 diabetes for all seven samples combined

rs no.	NCBI35 position	Alleles (1,2)	Additive		Additive (inverse variance)	
			OR (95% CI)*	*P**	OR (95% CI)†	*P*†
836	157781297	C,T	0.91 (0.83–1.01)	0.07	0.88 (0.78–0.99)	0.03
790055	157784219	A,G	0.96 (0.65–1.43)	0.83	0.90 (0.66–1.23)	0.50
2481080	157789410	C,T	0.93 (0.85–1.02)	0.10	0.89 (0.79–1.00)	0.06
2988726	157791827	A,G	1.01 (0.98–1.03)	0.61	0.91 (0.75–1.10)	0.33
2251597	157800920	C,G	1.10 (0.99–1.22)	0.06	1.15 (1.01–1.29)	0.03
2481084	157802687	C,T	1.10 (0.99–1.22)	0.07	1.14 (1.01–1.29)	0.04
7546890	157808465	A,G	0.91 (0.83–1.00)	0.06	0.90 (0.80–1.01)	0.06
11799478	157809827	C,T	1.11 (1.00–1.23)	0.06	1.15 (1.02–1.31)	0.02
4339888	157820399	C,G	1.09 (0.99–1.21)	0.09	1.14 (1.01–1.28)	0.04
3766383	157821645	G,T	1.18 (1.01–1.38)	0.03	1.14 (0.99–1.31)	0.06
3737787	157822596	A,G	1.06 (0.96–1.16)	0.23	1.10 (0.97–1.24)	0.13
2516840	157824390	C,T	1.05 (0.85–1.30)	0.65	1.04 (0.92–1.18)	0.50
2073656	157824428	C,G	0.97 (0.90–1.04)	0.35	0.93 (0.82–1.05)	0.23
2073653	157825833	A,G	1.17 (1.00–1.37)	0.05	1.13 (0.98–1.30)	0.08
2516839	157826194	A,G	1.11 (1.01–1.23)	0.04	1.14 (1.02–1.28)	0.02
2516838	157827443	C,G	0.92 (0.79–1.07)	0.27	0.94 (0.83–1.06)	0.29
1556259	157827722	C,T	0.88 (0.75–1.02)	0.09	0.90 (0.78–1.03)	0.12
2774279	157830629	A,G	1.07 (0.88–1.29)	0.49	1.04 (0.92–1.18)	0.49
3813610	157835712	C,G	1.10 (1.00–1.21)	0.05	1.11 (1.00–1.24)	0.05
7516231	157839152	A,T	1.24 (1.03–1.49)	0.02	1.17 (1.01–1.35)	0.04

Allele designations (1,2) are in line with Illumina convention (i.e., alphabetical for base name; see third column). **P* values based on assumptions of independence. Where appropriate (any cell with fewer than six observations), exact methods were used. OR and 95% CIs are calculated per copy of allele 1 (with reference to genotype 22) by modification of the method by Liu and Agresti (40). †*P* values estimated using inverse-variance method to take into account of lack of independence in the Amish sample. OR is calculated per copy of allele 1.

SNPs (from rs4339888 to rs1556259) showed no association with type 2 diabetes in any of the populations studied (online appendix Table 3).

Analyses combining data from these seven case-control comparisons are summarized in Table 2 (additive model only). At rs3737787, no evidence for association was observed under the additive (Table 2), dominant (for the rarer allele A: OR 1.08 [95% CI 0.93–1.25], $P = 0.32$), or recessive (1.15 [0.87–1.51], $P = 0.33$) models. Nominally significant associations under the additive model were seen for a cluster of highly correlated variants in *F11R* and for SNPs 3766383 and 2516839 within *USF1* (generating ORs between 1.10 and 1.15). With the exception of rs3766383 (model recessive for G, $P = 0.02$), combined analyses under the dominant and recessive models were not significant (data not shown).

Family-based association study. The same set of 19 SNPs were also typed in the full Pima dataset ($n = 832$). Case-control analysis (allowing for family structure) revealed no significant associations (online appendix Table 4). The most significant *P* value was seen at rs7516231 (MAF 0.33 in control subjects, 0.42 in cases, $P = 0.10$). At rs3737787, the equivalent allele frequencies were 0.35 (unaffected) and 0.33 (affected) ($P = 0.96$). The explicit test of within-family association was again nonsignificant for all SNPs (including rs3737787, $P = 0.22$).

Quantitative trait study. In the full set of 714 Amish subjects (631 with trait measures), there was no evidence of association with lipid or adiposity traits. For example, at rs3737787, there was no hint of association with lipid (triglycerides $P = 0.51$; HDL cholesterol $P = 0.80$) or anthropometric (BMI $P = 0.36$; waist-to-hip ratio $P = 0.10$; leptin $P = 0.19$) measures.

The Utah and Arkansas FSIGT subjects were genotyped separately for six tag SNPs. No associations with S_i, AIR_g, DI, or free fatty acid levels were seen among the 124 nondiabetic Utah family members. However, in this sample, several SNPs (rs2516838, rs2516839, and rs2516841) showed associations with cholesterol levels ($P = 0.001$, $P = 0.01$, and $P = 0.03$, respectively) when corrected for age, sex, BMI, and family membership (Table 3). Furthermore, SNPs rs2516839 and 3737787 were associated with triglyceride levels ($P = 0.03$ and $P = 0.007$, respectively). Crucially, however, it is the rare allele (A) at rs3737787 that is associated with raised triglycerides (in contrast with other reports [23,24]). In the second FSIGT sample (Arkansas), we found no significant associations with measures of S_i, β-cell function, glycemia, or lipids for any SNP.

Finally, we tested each of these six SNPs in the Utah case-control subjects with BMI, cholesterol, and triglyceride measures (116 case and 163 control subjects) after adjusting for BMI (in the lipid analyses), sex, and diabetes status. In contrast to the younger nondiabetic Utah family members, we found no association of any *USF1* SNP with cholesterol or triglyceride concentrations.

DISCUSSION

The main finding is that analysis of >3,700 subjects (using case-control and family-based association methods) found no reproducible evidence that variation within the *USF1* gene (or its neighbor *F11R*) is associated with type 2 diabetes. Though several nominally significant associations were found in the case-control study, these were not observed in the family-based association analysis. In any event, the variants displaying these associations have low prior odds for functional involvement (since none was strongly associated with FCHL) (23), and the estimated effect sizes are modest, particularly in the context of the 1q consortium's ongoing LD mapping efforts across the region, which have already identified many variants displaying far stronger replicated associations with type 2

TABLE 3
Metabolic traits by *USF1* genotype in Utah family members

SNP (rs)	Allele 1, 2	Model (with respect to allele 1)	1,1 (n)	Trait values for 1/1	1,2 (or combined group) (n)	Trait values for 1,2 (or combined group)	2,2 (n)	Trait values for 2,2	P value
Cholesterol (mmol/l)									
2516838	C,G	General	22	5.08 ± 0.19	60	4.29 ± 0.14	40	4.79 ± 0.15	0.001
		Dominant	22	5.09 ± 0.19	100	4.53 ± 0.11		—	0.010
		Recessive		—	82	4.45 ± 0.13	40	4.84 ± 0.16	0.047
2516839	A,G	General	52	4.96 ± 0.14	62	4.52 ± 0.14	11	4.23 ± 0.26	0.010
		Dominant	52	4.96 ± 0.14	73	4.55 ± 0.13		—	0.014
2516841	C,T	General	65	4.84 ± 0.13	45	4.45 ± 0.16	5	4.01 ± 0.37	0.026
		Dominant	65	4.83 ± 0.13	50	4.45 ± 0.15		—	0.033
Triglycerides (mmol/l)									
2516839	A,G	General	52	1.41 (1.18–1.69)	62	1.19 (0.99–1.43)	11	0.89 (0.63–1.24)	0.032
3737787	A,G	General	11	1.87 (1.31–2.66)	35	1.42 (1.17–1.73)	79	1.08 (0.90–1.28)	0.007
		Recessive		—	46	1.52 (1.27–1.82)	79	1.07 (0.90–1.26)	0.002

Data are marginal means ± SE or means (95% CI). Marginal means, adjusted for age, ln(BMI), sex, and pedigree membership are shown by genotype under general (three genotypes compared), dominant, and recessive models (with respect to allele 1 as designated in the second column). All values are in mmol/l. To convert to mass units, multiply cholesterol by 38.67 and triglycerides by 88.57. P values are shown for comparison of marginal means using the least significant difference test with 2 df under general and 1 df under dominant or recessive models. Triglycerides, which were ln-transformed for analysis, were converted back to a linear scale. Only statistically significant findings at $P < 0.05$ are shown.

diabetes (41). Crucially, the direction of these weak associations (favoring haplotypes carrying the more rare allele at rs3737787) is opposite to that reported in previous studies (23–27). While we cannot formally exclude the possibility that these nominal associations reflect true susceptibility effects (perhaps involving LD with other untyped variants), it is clear that previously reported type 2 diabetes susceptibility effects involving rs3737787 and other *USF1* variants (26,27) are not substantiated in this larger, more informative dataset in which cases were selected to be enriched for evidence of linkage to the *USF1* region.

In any study of this type, it is vital to be explicit about the extent to which the variants analyzed allow exclusion of effects at other untyped variants. In terms of capturing common variation in the *USF1* region, the 20 SNPs typed provide excellent coverage, as shown by their high density (close to one per kilobase in the *USF1* coding region), their documented capacity to tag hidden variation (at least in Europeans and East Asians), and by comparison to variants typed in previous studies (23,24,26). Recent evidence (42) suggests that rs2073658 has the best credentials as a functional etiological variant within *USF1*. Though we were unable to generate a working assay for this SNP, it is clear (from genotypes in Finns [23], French [26], and Caucasians [http://pga.mbt.washington.edu/]) that rs2073658 and rs3737787 are in almost complete LD (r^2 exceeding 0.99) in Europeans. LD patterns in the region suggest the same is likely to be true in the other populations studied. Thus, failure to type this SNP directly will have had minimal adverse effect on power. As with any indirect association study using common tag SNPs, we cannot exclude a role for rare variation within the gene (MAFs <5%) (43).

In terms of effect size, our study focused on populations with documented 1q linkage and preferentially sampled cases from multiplex families contributing to that linkage signal. Both maneuvers substantially boost power to detect variation contributing to the linkage signal (28,44). Taking these factors into account, we estimate that the current sample has (for $\alpha = 10^{-3}$, MAF of 10%, multiplicative model), 55% power for a variant with an allelic OR of 1.2 and 96% for an OR of 1.3. Given the relative power of linkage and association approaches when the etiological variants are typed (45), we can demonstrate, using reasonable assumptions, over 95% power to detect a variant contributing to at least 10% of the 1q linkage signal.

While the conclusions regarding *USF1* variation and type 2 diabetes seem clear, interpretation of the findings with respect to the other traits (especially lipid parameters) is less straightforward. In one sample (124 nondiabetic relatives of type 2 diabetic subjects from Utah), we did detect statistically strong associations with certain *USF1* SNPs (including rs3737787). However, the association between rs3737787 and triglycerides lies in the opposite direction to that described in Finns (23) and a different set of Utah pedigrees (24), and none of these associations could be replicated in the other samples (combined n = 1,091) or in a recent study of *USF1* variation in French subjects (28). The possibility should also be considered that *USF1* variants having modest effects on lipid parameters in other populations may be relatively less penetrant in the Amish, owing to the generally higher degree of physical activity in this population. While the analysis of lipid traits was not a major aim of our study, our data certainly suggest that the trait associations evident in several previous studies (23–27) are not reproducible in all other samples.

Despite the strong biological candidacy of *USF1* and its location within the well-replicated peak of linkage on chromosome 1q, we conclude, with confidence engendered by the large sample size, comprehensive capture of common variation within and around the gene (and the detailed analytical plan) that common variation within *USF1* has no discernible impact on type 2 diabetes susceptibility and that *USF1* makes no detectable contribution to the replicated linkage signal on chromosome 1q.

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