Original Article

Polymorphisms in the Glucokinase-Associated, Dual-Specificity Phosphatase 12 (DUSP12) Gene Under Chromosome 1q21 Linkage Peak Are Associated With Type 2 Diabetes

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Linkage of type 2 diabetes to chromosome 1q21-q23 is well replicated across populations. In an initial 50-kb marker map (580 markers) across the linked region, one of the two strongest associations observed in Utah Caucasians was at marker rs1503814 ($P < 0.00001$ in pools, $P < 0.004$ in individuals). Based on this association, we typed additional markers and screened for sequence variation in the nearby *DUSP12* gene. The strongest associations mapped to a highly conserved nongenic sequence just telomeric to rs1503814 and extended 10 kb telomeric through the *DUSP12* gene and into the 5′ end of the adjacent *ATF6* gene. No coding variant could explain the association in the *DUSP12* gene. An extended haplotype encompassing markers from −8,379 to +10,309 bp relative to the ATG start was more common in Caucasian case (0.381) than control subjects (0.285, $P = 0.005$) and was uniquely tagged by a 194-bp allele at either of two simple tandem repeat variants or by the T allele at marker +7,580. Markers −8,379 and +7,580 were nominally associated with type 2 diabetes in African-American subjects ($P < 0.05$), but with different alleles. Marker rs1503814 was strongly associated with postchallenge insulin levels among family members ($P = 0.000002$), but sequence variation in this region was not associated with type 2 diabetes in three other populations of European ancestry. Our data suggest that sequences in or upstream of DUSP12 may contribute to type 2 diabetes susceptibility, but the lack of replication suggests a small effect size. *Diabetes* 55:2631–2639, 2006

A genetic etiology underlying the high prevalence of type 2 diabetes is widely accepted. Nearly 30 genome scans and countless association studies have been conducted in multiple populations (1), which in aggregate and in recent meta-analyses (2) suggest that the genetic susceptibility to type 2 diabetes will involve many susceptibility loci. Genes identified to date have had small to moderate effects and have been difficult to replicate, and risk alleles have generally been marked by noncoding variants (2–5). Reported associations likely account for only a small portion of the total type 2 diabetes genetic susceptibility.

Linkage of type 2 diabetes to chromosome 1q21-q23 was described initially by our laboratory (6) in Northern European Caucasians and in Pima Indians (7), and it was subsequently replicated in British (8), French (9), and Amish Caucasians (1,10); in Chinese Han (1,11); and in supportive data in African Americans from Arkansas (S.C.E., unpublished observations). This well-replicated region of type 2 diabetes linkage is characterized by an extraordinary gene density, including a plethora of strong candidate genes. We recently reported that the region encompasses at least two and possibly three linkage peaks (12), and we reported previously that two other genes in this region were associated with type 2 diabetes (5,13). Both associations were observed in other populations (14–16), and they support a model of multiple susceptibility loci accounting for the chromosome 1 linkage signal.

In an initial collaborative effort to fine-map type 2 diabetes in Caucasians, we typed 580 single nucleotide polymorphisms (SNPs) over a 20-Mb region (mean 1 SNP/50 kb) that encompassed both major linkage peaks, using MassArray MALDI-TOF mass spectrometry (Sequenom, San Diego, CA) (5,17,18) in pooled DNA samples of 100 case and 100 control subjects of Northern European ancestry (5). We identified two prominent associations, one in intron 2 of the calsequestrin 1 gene (5) and a second at marker rs1503814 ($P < 0.00001$), which was verified by individual typing of the case and control subjects ($P =$

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AIR_g, acute insulin response to glucose; CNG, conserved nongenic region; FSIGT, frequently sampled intravenous glucose tolerance test; SNP, single nucleotide polymorphism; STR, simple tandem repeat.

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0.004). Based on these initial data, we evaluated additional SNPs from the public database across an 800-kb region surrounding the initial observation, and we narrowed the association to a 20-kb region that included dual-specificity phosphatase gene 12 (*DUSP12*) and the 5′ end of the endoplasmic reticulum stress factor *ATF6*. We report studies to identify the associated variants in this region in Caucasians and extend the analysis to African-American case and control subjects. We report that noncoding variants in and near the *DUSP12* gene, which has been shown to associate with and activate glucokinase in the liver (19), are associated with type 2 diabetes in both Utah Caucasian and Arkansas African-American populations, although a subset of the variants failed to replicate in other European populations.

RESEARCH DESIGN AND METHODS

DUSP12 gene variation was detected in 16 unrelated Caucasian individuals with type 2 diabetes from Utah families linked to the 1q21 region and 8 nondiabetic (control) family members, and it was detected in 16 diabetic members of African-American families used in the chromosome 1 linkage studies and 8 African-American control subjects. Association studies were conducted in 191 unrelated Caucasian case subjects and 188 unrelated Caucasian control subjects, as described previously (5,20). To reduce genotyping costs, some markers for initial genotyping were typed in pooled DNA samples, as described in detail previously (20,21). African-American samples were ascertained in Arkansas and included 130 unrelated nondiabetic individuals who had no family history of type 2 diabetes and 275 type 2 diabetic subjects who had at least 1 diabetic first-degree relative. Pooled DNA was constructed from 130 control individuals, 125 case subjects with type 2 diabetes and no nephropathy, and 150 individuals with type 2 diabetes and diabetic nephropathy (20). For markers more recently tested, we first typed 48 unrelated nondiabetic Caucasian and 48 nondiabetic African-American subjects for linkage disequilibrium before selecting markers for typing in the full population. Family-based association studies were conducted on 704 members of 68 Utah families used to define the original linkage, of which 292 individuals were considered affected (6,12). We tested the physiological impact of associated markers rs3820449 and simple tandem repeat (STR) marker −8379 in 120 individuals from the Utah families who had undergone tolbutamide-modified frequently sampled intravenous glucose tolerance tests (FSIGTs) (22) and an additional 182 Caucasian individuals from Arkansas who had undergone insulin-modified ($n = 78$) or tolbutamide-modified ($n = 104$) FSIGTs.

Subjects ascertained in Utah provided written informed consent under a protocol approved by the University of Utah institutional review board. Subjects studied in Arkansas provided written informed consent under protocols approved by the University of Arkansas for Medical Sciences human research advisory committee.

DUSP12 screening. We screened a total of 9,841 bp from each of the six exons, each intron, 2 kb of the 5′ flanking region, 481 bp of the 3′ flanking region, and the 2,634-bp conserved nongenic region (CNG) (Fig. 1) in 24 Caucasian and 24 African-American individuals, using denaturing high-performance liquid chromatography (Transgenomic, Omaha, NE) with bidirectional sequence analysis of fragments showing altered chromatographic patterns.

Genotyping of sequence variants. SNPs were typed, using pyrosequencing on a PSQ-96 (Biotage, Uppsala, Sweden) (5). SNPs at a distance from the initial observation were typed first in pooled Caucasian samples, and differences between case and control frequencies ≥5% were confirmed in individual samples. Three SNPs not amenable to typing by pyrosequencing were typed in individuals by oligonucleotide ligation (13). STR and insertion deletion markers were detected on LI-COR GR4200 sequencers and scored, using Gene Imager version 3.56 software (Scanalytic, Rockville, MD). For the noncoding regions, particularly in the DUSP12 5′ flanking region, which was not highly conserved, we first determined linkage disequilibrium and allele frequencies in 48 control samples. SNPs were chosen for typing in the full Caucasian and African-American populations based on a minor allele frequency over 5% and selection of tagged SNPs, using the parameter $r^2 > 0.95$ and the programs LDSelect (23) and TAGGER (32) (supplemental Table 1S, which is detailed in the online appendix [available at http://diabetes.diabetesjournals.org]). All SNPs included in this study were in Hardy-Weinberg equilibrium. Quality control steps included reliable automated calls by pyrosequencing software and inclusion of at least 30 duplicate samples for the full set. No assays failed the duplicate checks, and all variants typed in families showed Mendelian inheritance.

All members of families ascertained in Utah were typed for five DUSP12 variants: rs1503814 (−10473 bp), the −8379 bp STR (rs6143445), rs1027702 (−6735 bp), rs1063178 (+2115 bp), and rs3820449 (+7580 bp). African-American subjects were typed primarily for SNPs shown to be associated in Caucasian individuals, based on linkage disequilibrium determined in 48 unrelated African-American control samples. Additionally, we have typed >30 ancestrally informed SNPs as well as >70 total SNPs in candidate genes in the African-American population to detect admixture (24). These results did not support evidence for spurious association based on admixture among African-American subjects.

CNG. We searched the 20-kb region 5′ to the DUSP12 ATG start for potential CNGs that might represent regulatory sequences (25), using the May 2004 build (Build 35) and the University of California Santa Cruz conservation tracks (genome.ucsc.org) for human (hg17), chimp (panTro1), mouse (mm5), rat (rn3), and dog (canFam1). No conservation was observed in nonmammalian species (supplemental Fig. 2S). Multiple alignments were assigned a conservation score using phastCons (26), which is based on hidden Markov models, and MultiPipMaker (27).

Analysis of mRNA expression in transformed lymphocytes. Total RNA was isolated from Epstein-Barr virus–transformed lymphocytes, and allele-specific expression of SNP +2115 was quantified from 15 heterozygous individuals, as described previously (20,21). DUSP12 mRNA levels were measured from transformed lymphocyte RNA by quantitative real-time PCR (RT-PCR), using SYBR Green, and normalized to 18S RNA.

Typing of International Type 2 Diabetes Chromosome 1q Consortium samples. We tested for replication of SNPs in the DUSP12 region in the Caucasian subset of the International Type 2 Diabetes Chromosome 1q Consortium samples, including 356 of the samples included in the Utah studies reported here (191 case and 165 control subjects), 504 samples from the Amish Family Diabetes Study (150 case and 354 control subjects) (10), 547 samples from a French diabetes study (259 case and 288 control subjects) (9), and 890 samples from the U.K. (443 case and 447 control subjects) (8). Genotyping was performed as part of a high-throughput, large-scale dense mapping effort on chromosome 1q of three bundles of 1,536-plex assays, using the Illumina Golden Gate assay (28) performed at the Wellcome Trust Sanger Institute, that included SNPs noted to be associated in individual populations. The International Type 2 Diabetes Chromosome 1q Consortium map included 13 SNPs covering the region from 158,436,917 to 158,461,856 bp on chromosome 1q (the region encompassing DUSP12). SNPs rs1417580 (+10157 in the Utah study) and rs12756470 (not typed in the Utah study) failed quality control steps in this multiplex assay and were excluded. Quality control steps included blinded duplicates, analysis of plate-to-plate variation, and adherence to Hardy-Weinberg equilibrium. A subset of Arkansas African-American samples was represented in the International Type 2 Diabetes Chromosome 1q Consortium typing, but we report only the non-Consortium typing of the full African-American case-control set.

Statistical methods. Allele frequencies were compared, using the χ^2 or Fisher's exact tests or, in the case of the multiallelic STR polymorphisms, the CLUMP program (29). We considered $P < 0.05$ to be significant, and we report uncorrected P values. Secondary analyses of genotypic association were computed, using Pearson's χ^2 test and logit regression models under additive, dominant, and recessive models. Pairwise linkage disequilibrium was estimated from the combined case and control population data, using the expectation maximization algorithm, from which D′ and r^2 were calculated, using HaploView 3.2 (30). Extended haplotypes were estimated, using PHASE 2.1.1 or HaploView 3.2 programs (31,32). Insulin sensitivity index (S_i) and acute insulin response to glucose (AIR_g) were calculated from FSIGT data, using the MinMod program (33) as described previously (22). Genotype effects on insulin secretion and insulin sensitivity were tested using general linear models with age, sex, BMI, glucose tolerance status, and family membership for Utah families as covariates and factors (22). For unrelated individuals from Arkansas, we included the protocol (tolbutamide or insulin) as a covariate (34). Family-based association was calculated by a maximum likelihood implementation of the transmission disequilibrium test computed in the Pedigree Analysis package (13,35) and conducted on the full set of linkage families, including families not showing linkage to chromosome 1. Family-based quantitative trait associations were tested, using the Pedigree Analysis package (36), by comparing the maximized likelihood estimation of the three genotypic means to that if the three means were equal. A Pearson's χ^2 test statistic with 2 degrees of freedom (df) was computed as twice the natural logarithm of the ratio of the two likelihoods. Familiality was accounted for through a polygenic component, and sex, age, and BMI effects were estimated. For both insulin and glucose measures, only nondiabetic individuals were examined. Insulin and glucose values were also examined as multivariate values, using all five oral glucose tolerance test time points simultaneously (10

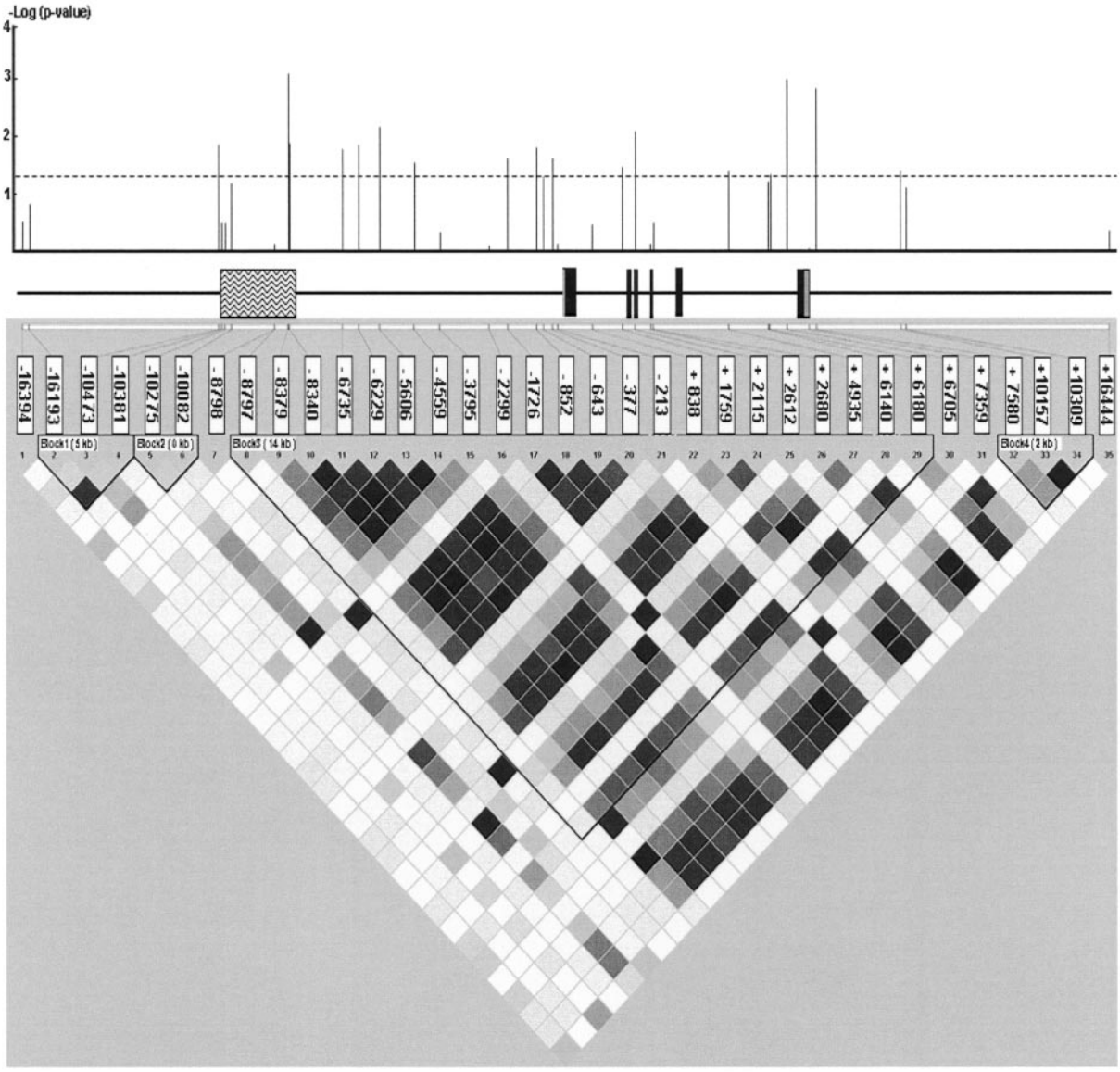

FIG. 1. DUSP12 region map in Caucasians. The CNG is shown as a shaded box, exons 1–6 as darkened blocks, and untranslated regions as lighter shaded boxes. The block structure based on CIs of D′ is outlined as triangles, and the shaded blocks represent linkage disequilibrium measured as r^2 between pairs of variants. For purposes of this figure, both STR polymorphisms (−8379 and +6705) were dichotomized to 194 versus not 194 alleles, as described in the text. Bars above the figure are based on negative log of *P* values for tests of allelic association.

df) while allowing for correlations between them. This method included individuals for whom some time points were missing. Transcription factor binding sites were predicted using the TFSEARCH version 1.3 program, which is based on the TRANSFAC database (37).

For analysis of International Type 2 Diabetes Chromosome 1q Consortium data, between-group differences in allele frequency were evaluated on a population-specific basis, using standard contingency table methods, and exact *P* values were calculated using Stata SE version 8 (Stata, College Station, TX). Single-point data from the case-control samples were combined, using the Mantel-Haenszel fixed-effects method (Stata SE version 8), and combined odds ratios were generated under dominant and recessive models. Analyses in the Amish took into account correlations among related individuals.

RESULTS

Analysis of DUPS12 region in Caucasians. Based on the initial observation of an association at SNP rs1503814 (location 158,440,777 bp), we selected SNPs for 5-kb spacing extending from 158,416,548 to 158,897,701 bp and identified 48 SNPs that could be typed successfully (mean interval 5.1 kb), including 7 SNPs extending 24.2 kb centromeric and 41 SNPs telomeric to SNP rs1503814 (supplemental Table 1S). The associated SNPs clustered primarily in the 48-kb region telomeric to rs1503814 (158,440,777 to 158,488,694 bp; May 2004 Build 35) and encompassing the DUSP12 gene, a widely expressed dual-specificity phosphatase with homology to the yeast gene YVH1 (38) that has been shown to regulate glucokinase (19). The associated SNPs spanned a region of high sequence conservation among all mammalian species (CNG) between 158,440,995 and 158,443,222 bp (supplemental Fig. 2S), the 3′ boundary of which was 8,017 bp upstream from the DUSP12 ATG start (Fig. 1). We screened the full region from −10 kb upstream (a highly conserved, nongenic region) to 10 kb downstream of the DUSP12 ATG start site (Fig. 1). We identified 47 SNPs, including 7 SNPs previously typed (supplemental Table 1S), and, based on the observed associations in Caucasians (Table 1 and Fig. 1), extended the screening to 24

TABLE 1
Summary of SNPs typed in Caucasian individual samples for DUSP12 region

Location	Name	Alleles	Case frequency	Control frequency	*P* allelic	*P* additive	*P* recessive	*P* dominant
−34702	rs905595	G/C	0.255	0.250	0.704	0.900	0.830	1.00
−32767	rs2050887	A/G	0.042	0.034	0.356	0.700	1.00	0.700
−27665	rs905591	C/G	0.121	0.139	0.526	0.690	0.450	0.630
−23504	rs1417582	G/A	0.288	0.312	0.431	0.092	0.37	0.121
−22594	rs1875763	C/G	0.516	0.481	0.269	0.500	0.290	0.730
−16394	rs905589	C/T	0.356	0.389	0.301	0.640	0.460	0.460
−16193	rs905590	T/C	0.042	0.064	0.155	0.340	0.500	0.240
−10473	rs1503814	A/G	0.234	0.314	0.015	0.039	0.180	0.022
−10381	rs12021510	T/C	0.053	0.070	0.365	0.664	1.00	0.430
−10275	rs17415059	G/A	0.379	0.414	0.333	0.398	1.00	0.460
−10082	Novel	1 bp Del/Ins	0.166	0.219	0.067	0.154	0.33	0.085
−8798	rs6657266	G/A	0.052	0.057	0.978	ND	ND	0.870
−8797	rs17415066	C/T	0.073	0.067	0.790	ND	0.24	0.550
−8379	rs6143445	STR (x/194)	0.297	0.196	0.0008	0.0003	1.00	0.00011
−8340	rs1340981	T/C	0.437	0.356	0.0134	0.056	0.041	0.106
−6735	rs1027702	C/T	0.428	0.351	0.0172	0.092	0.14	0.052
−6229	rs10917821	C/G	0.377	0.306	0.0143	0.092	0.119	0.077
−5606	rs4657096	G/A	0.446	0.356	0.0072	0.036	0.036	0.052
−4559	rs2174208	C/T	0.429	0.358	0.0293	0.078	0.038	0.196
−3795	rs6687091	C/T	0.388	0.413	0.490	0.681	0.440	1.00
−2299	rs10917835	T/A	0.053	0.054	0.844	ND	ND	1.00
−1726	Novel	CT Ins/Del	0.407	0.337	0.027	0.037	0.743	0.013
−852	rs6427625	C/G	0.432	0.36	0.016	0.113	0.140	0.083
−643	rs6427626	G/T	0.479	0.415	0.052	0.213	0.248	0.147
−377	rs12121310	A/C	0.379	0.308	0.026	0.096	0.166	0.06
−213	rs953301	G/A	0.044	0.048	0.795	ND	ND	0.715
838	Novel	C/G	0.291	0.321	0.377	0.427	1.00	0.25
1759	rs7540096	A/G	0.495	0.418	0.035	0.105	0.159	0.068
2115	rs1063178	G/A	0.382	0.291	0.008	0.023	0.123	0.012
2612	rs1063179	G/A	0.051	0.059	0.788	ND	ND	0.623
2680	rs10917852	G/T	0.377	0.411	0.344	0.445	1.00	0.278
4935	rs12564653	T/C	0.484	0.410	0.042	0.118	0.194	0.071
6140	rs12026369	C/G	0.032	0.060	0.062	ND	ND	0.071
6180	rs4656332	G/A	0.43	0.359	0.048	0.133	0.192	0.084
6705	rs10527814	STR (x/194)	0.326	0.218	0.001	0.0002	0.00007	0.85
7359	rs16863049	C/G	0.045	0.044	0.946	0.595	ND	1.00
7580	rs3820449	C/T	0.294	0.194	0.0015	0.00018	1.00	0.0001
10157	rs1417580	G/T	0.482	0.407	0.043	0.102	0.192	0.056
10309	rs1417581	G/C	0.476	0.413	0.082	0.175	0.299	0.094
16444	rs2298019	G/A	0.130	0.148	0.4794	0.769	0.682	0.558

SNPs that were in complete linkage disequilibrium ($r^2 = 1$) were typed only in 48 control samples and are not included. Likewise, SNPs with low minor allele frequencies (<5%) were generally not tested. Raw counts are available in supplemental Table 2, which is detailed in the online appendix. *P* values are shown for allelic tests, additive genotypic model (*P* additive), rare allele recessive model (*P* recessive), and rare allele dominant model (*P* dominant). ND, not determined where rare allele was uncommon and hence homozygotes were not observed.

African-American individuals. Among Caucasians, 19 variants were associated with type 2 diabetes ($P < 0.05$), clustered primarily between −8379 and +7580 bp relative to the DUSP12 ATG start site (15/19 SNPs) and encompassing the DUSP12 gene and 5′ flanking region (Fig. 1). An additional six variants showed a trend to an association with type 2 diabetes ($P < 0.1$), of which three again fell in this interval. Based on these results, we focused the study on the region from −16 to +16 kb. The data are summarized in Table 1 and graphically in Fig. 1, and raw genotype counts are shown in supplemental Table 2S. Based on the confidence interval (CI) block definition (39), the entire DUSP12 gene, the 5′ promoter, part of the CNG, the 3′ flanking region to +6180 bp, and most associated variants were encompassed in one block (block 3) (Fig. 1 and supplemental Fig. 1S). Most associated SNPs that fell outside of this interval were nonetheless in linkage disequilibrium with block 3 SNPs by r^2 (Fig. 1). However, rs1503814 (−10473), which was the original observation, fell outside of the associated block and was not in strong linkage disequilibrium with the most significantly associated variants.

The associated region included two STR variants at −8379 and +6705. We tested the association in Caucasians using the CLUMP program (29) (supplemental Tables 3S and 4S); for both STRs, the 194 allele was significantly overrepresented in case subjects relative to control subjects. Using the T3 test, uncorrected *P* values were 0.0013 and 0.0019 for STRs −8379 and +6705, respectively, with *P* values based on Monte-Carlo simulation of 0.011 and 0.023, respectively. To facilitate further analyses, we dichotomized both STRs to 194 versus X, where X was any other size. In post hoc analyses, we tested dominant and recessive models for all SNPs and for dichotomized STRs.

TABLE 2
DUSP12 region haplotypes in Caucasians

Haplotype	Case frequency	Control frequency	χ^2	P
2TCCGCC2CGAGAGTTG**2**CGG	0.277	0.310	0.957	0.328
1CTGATT1GTCCGAGCA**1**TTC	0.271	0.184	7.964	0.005
2TCCGCT2CGACAGGTG**2**CGG	0.124	0.168	2.907	0.088
2CTGATT1GTCCGAGCA**2**CTC	0.084	0.094	0.203	0.652
2TCCGCC2CGACAGTTG**2**CGG	0.076	0.069	0.145	0.703
2TCCGCT2CTACGGGCA**2**CTC	0.043	0.063	1.515	0.218
2CTCATT1GTACGGGCG**2**CTC	0.040	0.042	0.023	0.879
2TCCGCC2CGACAGTTG**1**CGG	0.014	0.025	1.136	0.287

Table 2 shows the haplotypes from the STR at −8379 through SNP +10309, with only the common (minor allele frequency >0.1) variants included. The two STR variants (in bold) are encoded as 194 bp = 1, and all other alleles = 2 (−8379 and + 6705). For the InDel variant at −1726, deletion is encoded as 1 and insertion as 2. The table includes 21 variants: −8379, −8340, −6735, −6229, −5606, −4559, −3795, −1726, −852, −643, −377, +838, +1759, +2115, +2612, +2680, +4935, +6180, +6705, +7580, +10157, and +10309.

The associated sequence variants were most consistent with a dominant or additive model for the minor allele (Table 1 and supplemental Table 2S).

Haplotype analysis in Caucasians. We first analyzed the haplotypes for each of the four blocks defined by D′ CIs (39) (Fig. 1 and supplemental Fig. 1S), using the PHASE 2.1 program to permit analysis without recoding of the STR alleles (32). In block 2, the three-marker haplotype −10473, −10381, and −10275 was associated with type 2 diabetes ($P = 0.04$ for global; $P = 0.0018$ for combination ATG present at 0.381 in case subjects and 0.285 in control subjects). Block 3 (supplemental Fig. 1S) extended from −8379 to +6180 and encompassed 16 variants, of which 11 showed nominal association ($P < 0.05$). However, visual inspection suggested an area of linkage disequilibrium extending from SNP −8798 to SNP +10309 (supplemental Fig. 1S) and encompassing 23 variants. Hence, we repeated the haplotype analysis with all variants with minor allele frequency >0.1. A single haplotype was overrepresented in case subjects (0.261) versus control subjects (0.179), was the most common single haplotype (22 vs. 5% for the next most common), and was uniquely tagged by the 194-bp allele at either the −8379 or +6705 STR or by the T allele at SNP 7,580 bp (rs3820449) (supplemental Table 3S). When we again dichotomized both STRs (194 vs. X, as noted above), only eight haplotypes were observed. The single-risk haplotype differed from the other two common (frequency over 10%) haplotypes at every position (Table 2). Similar conclusions were reached when haplotypes were constructed from a five-variant moving window (supplemental Table 6S). The most significant ad hoc SNP combination, which included SNPs from three blocks but excluded the STRs, was −10473, −8379, +2115, and +7580 (global $P = 0.00015$).

Association and haplotype analysis in African Americans. To test for replication and to narrow the associated region, we examined 27 SNPs (−10473 to +192519) in 48 African-American individuals (supplemental Figs. 3S and 4S). We selected 16 SNPs and the −8379 STR for minor allele frequencies >5% and based on linkage disequilibrium ($r^2 < 0.95$) to test in the full sample (Table 3). Variants −8379 and +7580 were nominally associated with type 2 diabetes, but in both case subjects, the allele frequencies and associated alleles differed from Caucasians (Fig. 2 and supplemental Table 4S). We observed 12 alleles at −8379 (from 194 to 224 bp), which showed nominal association with type 2 diabetes: CLUMP analysis (29); T2 and T3 tests $P = 0.049$ and $P = 0.011$, respectively; empirical P values of 0.079 and 0.070, respectively, with 10,000 replicates. At SNP +7580 (Table 3), the minor T allele was 10-fold less common among African Americans (0.03 in type 2 diabetic case subjects) than among Caucasian case subjects; the major C allele was overrepresented in type 2 diabetes. Only 2 of 14 haplotypes for the block including DUSP12 showed a nominal association ($P < 0.05$) with type 2 diabetes (haplotypes 3 and 10) (supplemental Table 7).

Metabolic and family analyses of DUSP12 variants in Caucasians. We tested the −8379 STR and SNP +7580 in 120 members of 26 Utah families (22). As observed in the larger population, the −8379 194-bp allele was found only in individuals with the +7580 T allele; thus, we classified the sample as 194-T homozygotes; 194-T/X-C heterozygotes, where X was any other STR allele; and X/C homozygotes. Marginal means did not differ by genotype for insulin sensitivity (S_i), AIR_g, or disposition index ($S_i \times AIR_g$). In a second population of 182 unrelated glucose-tolerant Caucasian individuals from Arkansas (96 homozygous for the low-risk haplotype, 77 heterozygous, and 8 homozygous for the high-risk haplotype), we likewise found no evidence for an effect of DUSP12 haplotype on S_i, AIR_G, or disposition index.

We used SIMWALK 2 (40) to estimate haplotypes for markers −10473, −8379, −6375, +2115, and +7580 in 740 members of the previously described Utah families (5,6). No variant or haplotype showed excess transmission from parents to affected offspring (5,13), even when restricted to the specific families that generated the linkage signal in this region (6,12). Furthermore, the −8379 194-bp allele did not explain the linkage signal, using the GIST (Genotype-IBD Sharing Test) (41). In contrast, the −10473 SNP was significantly associated with fasting ($P = 0.018$), 30-min ($P = 0.02$), 60-min ($P = 0.014$), and 120-min ($P = 0.001$) insulin, and particularly the multivariate measure combining all five postglucose challenge insulin levels ($P = 0.000002$). SNPs +2115 and +7580 showed a similar albeit lesser association with the multivariate insulin measure ($P < 0.01$). No SNP was associated with fasting or postchallenge glucose levels, however.

Transformed lymphocytes were available from most family members. Among 15 individuals heterozygous for transcribed SNP +2115, equal amounts of cDNA were observed from both alleles, and total DUSP12 message levels did not differ between 21 T/T homozygotes and 8 C/C homozygotes (DUSP12–to–18S ratios 9.84 ± 8.73 T/T

TABLE 3
Summary of variants fully typed in African-American case and control subjects

Location	Major/minor allele	Case minor allele frequency	Control minor allele frequency	χ^2	P
−10473	A/G	0.382	0.387	0.016	0.900
−8379	208 + 214/X	0.326	0.398	4.44	0.035
−8340	C/T	0.166	0.197	1.31	0.250
−6735	T/C	0.178	0.193	0.30	0.580
−5606	A/G	0.198	0.210	0.24	0.620
−643	T/G	0.157	0.175	0.56	0.450
838	C/G	0.049	0.071	2.25	0.130
4891	C/T	0.173	0.160	0.28	0.590
6557	T/A	0.347	0.302	2.27	0.130
7359	C/G	0.316	0.310	0.04	0.840
7580	C/T	0.033	0.062	4.06	0.044
10309	C/G	0.157	0.177	0.68	0.410
21683	A/G	0.134	0.172	2.92	0.088
32150	A/G	0.047	0.060	0.84	0.360
41684	C/G	0.056	0.057	0.01	0.930
41720	C/T	0.149	0.126	1.10	0.290
209180	C/T	0.373	0.383	0.12	0.730

Variants typed in African-American families are shown by location, as in Table 1 and Fig. 1. Major and minor alleles are switched in African Americans from Caucasian frequencies for some SNPs. Note that −8379 has been dichotomized for this table into 208- and 214-bp alleles versus all others. The actual distribution is shown in Fig. 3. χ^2, the χ^2 value for allelic association (1 df); P value, the P value from χ^2 test.

vs. 9.59 ± 3.17 C/C).

Replication in other chromosome 1q–linked populations. To determine whether *DUSP12* SNPs were associated with type 2 diabetes in other populations, we examined three additional populations of European ancestry with linkage to the 1q21-q23 region: Amish (150 case and 354 control subjects), French (159 case and 288 control subjects), and British (443 case and 447 control subjects) Caucasians. Although nominal associations were noted at SNPs +2115 and +7580 ($P < 0.05$) on meta-analysis of all populations of European ancestry, no association was found when Utah samples were excluded ($P >$ 0.3). Genotype data are shown in supplemental Table 8S.

DISCUSSION

Chromosome 1q21-q23 is among the best-replicated regions of linkage to type 2 diabetes, with evidence for linkage in multiple Caucasian populations, Pima Indians, Chinese, and, in unpublished studies from our laboratory, African Americans. Previous studies from our laboratory strongly support the existence of multiple susceptibility genes contributing to the overall linkage signal and possibly to the replication of linkage on 1q21-q23. Our current

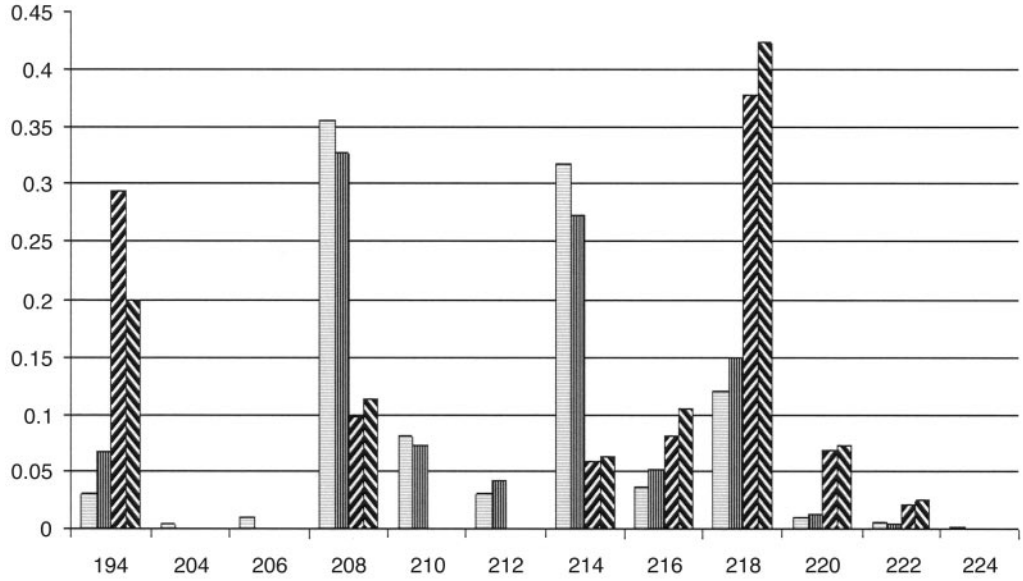

FIG. 2. Allele distribution in Caucasian and African-American case and control subjects at STR −8379. DUSP12 −8379 STR shows the large differences in allele frequencies by ethnicity. Numbers are shown as the frequency of each allele. Total number of chromosomes were 532 for African-American diabetic subjects, 314 for African-American control subjects, 372 for Caucasian diabetic subjects, and 362 for Caucasian control subjects. ▤, African-American case subjects; ▥, African-American control subjects; ▨, Caucasian case subjects; ▧, Caucasian control subjects.

studies have focused on the largest linkage peak, centered at 158 Mb, encompassing markers *CRP* and *ApoA2*, and including the calsequestrin 1 gene (*CASQ1*; 157 Mb), for which we (5) and Fu et al. (14) recently reported an association. That association seemed unlikely to account for the entire linkage signal, and in the current study we followed up on an association at SNP rs1503814, 1.4 Mb away from the *CASQ1* gene.

This region included only three genes. *FCRLM2*, which is 5′ to rs1503814 and in a region without associated SNPs, is a member of the Fc receptor family involved in antibody-dependent cell cytotoxicity. The dual-specificity phosphatase 12 (*DUSP12*) gene is widely expressed and shows broad conservation even in yeast. Indeed, the human *DUSP12* gene can complement a yeast mutant (38). The entire gene and most of a 5′ CNG were included in a single haplotype block (Fig. 1), and within this block most of the common variants were associated with type 2 diabetes in Utah Caucasians (Fig. 1 and Table 1). Although we found some evidence among Caucasians for associated SNPs in the 5′ end of the *ATF6* gene, these were clearly outside the *DUSP12* haplotype block and not in linkage disequilibrium with the *DUSP12* SNPs (data not shown). ATF6 is an essential activator of endoplasmic reticulum stress and the unfolded protein response (42) and, based on recent data suggesting a role of endoplasmic reticulum stress in type 2 diabetes, insulin resistance, and impaired insulin secretion (43), a strong functional candidate. Work is in progress to fully evaluate the large *ATF6* gene, but we focused the current study on *DUSP12* as the more probable candidate based on the clustering of associated SNPs and STRs, the CNG, and evidence for association (albeit with different alleles) in African-American subjects.

DUSP12 was pulled from a rat hepatic cDNA library with a yeast two-hybrid system, using glucokinase as bait, and identified as the glucokinase-associated protein (19). DUSP12 (glucokinase-associated protein) accelerated glucokinase activity in a manner suggesting functional significance. Hence, DUSP12 may modulate glycolysis in the liver and pancreatic β-cell through dephosphorylation of glucokinase in the cytoplasm. The wide distribution and relatively high levels of expression suggest that DUSP12 may have other, as yet unidentified, roles. Because we identified no coding variants that could explain the association in DUSP12, we propose that the associated variant or variants, mainly in highly conserved regions, may be regulatory. The −8379 STR, which showed strong associations in Utah Caucasians and more modest associations in African Americans, is within a very highly conserved region of >2 kb, 8 kb upstream of *DUSP12* (supplemental Fig. 2S). The 194 allele, which was associated with type 2 diabetes in Caucasians, eliminates the binding site for the transcription factor SRY (sex-determining region Y), but this factor appears unlikely to have a role in type 2 diabetes. Based on the strong conservation across mammalian species, this region may act as an enhancer for *DUSP12*, *ATF6*, or a chromosome 1 gene beyond this region of association. Given the size of the CNG, we considered that the region might be transcribed, but we were unable to detect a transcript in HepG2 cells or transformed lymphocytes (unpublished data). The +6705 microsatellite is not in a conserved region, but it contains multiple copies of the predicted binding site for the homeobox transcription factor CDXA (CDX1; caudal type homeobox transcription factor 1), which is widely expressed. The multiple CDXA binding sites and lack of conservation suggest that the +6705 variant may not itself be the causative variant. In contrast, SNP +7580, at the 3′ end of DUSP12, is at the downstream end of a region of conservation among mammals. The common C (G) allele is conserved in dogs, chimpanzees, and humans, and it binds the basic helix-loop-helix transcription factor TH1/E47. That factor in turn interacts with key pancreatic β-cell genes to regulate insulin gene transcription. Altered binding might impact transcription of either *DUSP12* upstream or *ATF6* downstream in a tissue-specific manner. However, opposite alleles are associated in Caucasians and African Americans, which appears inconsistent with a causative variant. Finally, although STR −8379 and SNP +7580 show the strongest associations with type 2 diabetes and are in the most conserved regions among mammals, the strongest quantitative trait association was with SNP −10473 farther upstream and post–glucose load insulin levels. This SNP was the location of the original observation, but it was no longer the strongest association with type 2 diabetes once additional case and control subjects were typed.

Replication of association with complex disease genes has been difficult, and, consequently, concerns have been raised about the power to detect and replicate complex disease associations (44). Multiple apparently convincing associations in smaller samples have failed to replicate in very large populations (45–47). Both of our study populations are relatively small, although our previously described association at the *PKLR* locus has held up in the larger International Type 2 Diabetes Chromosome 1q Consortium data set (16). Thus, the associations found here may well be spurious. Facts favoring a role for *DUSP12* in type 2 diabetes include the strong candidacy based on a previous study (19); the finding of associated *DUSP12* region markers in a second population, albeit with less significance and with different alleles; and the association of SNPs in this region with postchallenge insulin levels in unaffected family members.

Nonetheless, several aspects of our extensive analyses did not support a direct role of *DUSP12*. First, we could not find a difference in allelic expression in transformed lymphocytes for a marker (SNP +2115) that is in strong linkage disequilibrium with the risk haplotype, nor did *DUSP12* expression differ among homozygotes at this SNP. This finding is inconsistent with a *cis*-acting regulatory variant, and may suggest involvement of a gene other than *DUSP12*. Alternatively, altered DUSP12 regulation may be tissue specific. Second, the only physiologic consequence of *DUSP12* region variants was on the insulin response to an oral glucose load, but detailed measures of insulin sensitivity and insulin response to intravenous glucose were not altered. Other challenges, such as a graded glucose infusion or measurement of hepatic glucose function, may be required to understand the physiologic importance of *DUSP12* variants. Third, the associated variants were not transmitted in excess from parents to affected offspring, nor could we explain the linkage signal using the GIST program. Such tests have also failed for *PKLR* alleles and *CASQ1* variants (5,13). For variants with a minor allele frequency of 0.2, using all available Utah families, we have 63% power to detect a transmission rate of 0.6 from a heterozygous parent to affected offspring, which translates to a relative risk of 1.24 under a dominant and 1.37 under a recessive model. Finally, and perhaps most importantly, analysis of SNPs in this region, including the associated SNP +7580, showed

no association with type 2 diabetes in the European-based populations from the International Type 2 Diabetes Chromosome 1q Consortium when Utah samples were not included. Notably, these studies did not include the two STR variants that show the strongest associations in the Utah population. Although typed SNPs, particularly +7580 (rs3820449), should have served as a proxy for the 194-bp allele, the possibility remains that these studies did not type the functional variants.

In summary, we have performed a detailed analysis of markers in a ~20-kb region surrounding the *DUSP12* and *ATF6* genes. We report evidence for an association with type 2 diabetes in Utah Caucasians, with some evidence for association in African Americans recruited in Arkansas, albeit with different alleles. The most strongly associated SNPs are in highly conserved regions, thus suggesting a possible regulatory role. SNPs in this region are also associated with insulin levels after an oral glucose challenge. However, attempts to replicate the SNP associations in a meta-analysis of data from the three other European populations with linkage of type 2 diabetes to chromosome 1q21-q23 showed no significant association. Interactions between *DUSP12* region SNPs and other chromosome 1 variants or variants elsewhere in the genome might reconcile these differences, as might the typing of the STR variants in other populations. Additional studies will be needed to determine whether the associations observed in Utah Caucasians are spurious or play a role in insulin response to glucose and susceptibility to type 2 diabetes.

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