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A Genome-Wide Association Study in American Indians Implicates *DNER* as a Susceptibility Locus for Type 2 Diabetes

Most genetic variants associated with type 2 diabetes mellitus (T2DM) have been identified through genome-wide association studies (GWASs) in Europeans. The current study reports a GWAS for young-onset T2DM in American Indians. Participants were selected from a longitudinal study conducted in Pima Indians and included 278 cases with diabetes with onset before 25 years of age, 295 nondiabetic controls ≥45 years of age, and 267 siblings of cases or controls. Individuals were genotyped on a ~1M single nucleotide polymorphism (SNP) array, resulting in 453,654 SNPs with minor allele frequency >0.05. SNPs were analyzed for association in cases and controls, and a family-based association test was conducted. Tag SNPs (n = 311) were selected for 499 SNPs associated with diabetes ($P < 0.0005$ in case-control analyses or $P < 0.0003$ in family-based analyses), and these SNPs were genotyped in up to 6,834 additional Pima Indians to assess replication. Rs1861612 in *DNER* was associated with T2DM (odds ratio = 1.29 per copy of the T allele; $P = 6.6 \times 10^{-8}$, which represents genome-wide significance accounting for the number of effectively independent SNPs analyzed). Transfection studies in murine pancreatic β-cells suggested that *DNER* regulates expression of notch signaling pathway genes. These studies implicate *DNER* as a susceptibility gene for T2DM in American Indians.

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A number of genetic variants associated with type 2 diabetes mellitus (T2DM) have been identified (1–6). Since most established susceptibility variants were detected by genome-wide association studies (GWASs) in Europeans, GWASs in non-European populations may identify other important variants. Except for our preliminary study with 80,044 markers in Pima Indians (7), there are few GWASs for T2DM in American Indians. In the current study, we extend this GWAS to include 453,654 single nucleotide polymorphisms (SNPs), and we genotype 6,834 additional individuals to identify reproducibly associated variants.

RESEARCH DESIGN AND METHODS

Participants

Participants were derived from a longitudinal study conducted in the Gila River Indian Community (8). Diabetes was diagnosed by a 75 g oral glucose tolerance test

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according to 1997 American Diabetes Association criteria (9) or if the diagnosis was made during routine clinical care. As described previously, participants informative for analyses of young-onset diabetes or for metabolic traits related to diabetes were selected for the GWAS (10). All individuals were full-heritage American Indian, and they included 278 "cases" who developed diabetes before age 25 years (mean ± SD age of onset = 19.4 ± 4.4 years) and 295 "controls" who were nondiabetic and ≥45 years old when last examined (mean age = 55.2 ± 9.7 years). To allow for family-based analyses, discordant siblings were included. These included 129 nondiabetic siblings of cases last examined at an age older than the case's age of onset (mean age = 28.1 ± 8.2 years) and 138 diabetic siblings of controls who developed diabetes when they were younger than the age when the control was examined (mean age at diagnosis = 40.8 ± 8.4 years). The diabetes GWAS involved 840 individuals in 514 sibships.

Replication studies were conducted in additional individuals from the longitudinal study. Initial studies (replication set 1) were conducted in 2,908 individuals (mean age = 40.0 ± 16.3 years; 43.8% with diabetes) who were not part of the GWAS and who were full-heritage Pima or who had data on metabolic traits. Selected SNPs were further typed in a second group (replication set 2) of 3,926 individuals who were largely "mixed" heritage (mean age = 27.7 ± 13.9 years; 20.2% with diabetes; mean Pima heritage = 55%). Supplementary Table 1 shows characteristics of the groups. Studies were approved by the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases.

Association of diabetes-associated SNPs with metabolic characteristics of T2DM was assessed. Maximum BMI observed in the longitudinal study was analyzed in 6,786 individuals examined when ≥15 years of age. Fasting serum insulin concentration was measured in 5,400 of these individuals when they were nondiabetic; measures of insulin resistance (homeostasis model assessment of insulin resistance [HOMA-IR]) and β-cell function (homeostasis model assessment of β-cell function [HOMA-B]) were calculated (11). Detailed physiologic measures were made in 400 nondiabetic full-heritage Pimas. Percentage of body fat was calculated by hydrostatic weighing or dual X-ray absorptiometry, and insulin sensitivity was determined by the hyperinsulinemic–euglycemic "clamp" (12). Acute insulin response, 3–5 min after a 25 g intravenous glucose bolus, was measured in 288 normoglycemic individuals (12).

Genotyping

Genotypes in the GWAS were generated on the Affymetrix 6.0 Human SNP Array (Affymetrix, Santa Clara, CA) using the BIRDSEED algorithm, as described previously (10). SNPs were excluded if >15% of genotype calls were missing, if genotype frequencies diverged from Hardy–Weinberg expectations ($P < 0.001$), if concordance among 100 duplicate samples was <97%, or if minor allele frequency was <5%. Thus 453,654 SNPs were analyzed. Supplemental Fig. 1 shows the selection of SNPs in different samples.

Genotyping in replication studies was performed by BeadXpress (Illumina, San Diego, CA) or Assays-on-Demand (Applied Biosystems, Carlsbad, CA) according to manufacturer's protocol. To confirm accuracy of initial GWAS genotypes, all GWAS participants were retyped for each SNP in replication studies, and these genotypes were used in subsequent analyses. Forty-five SNPs with large differences in allele frequency between American Indian and European populations (13) were genotyped for estimation of the proportion of European heritage, utilizing the method of Hanis et al. (14), for use as a covariate.

Statistical Analysis

Association between young-onset T2DM and each SNP in the GWAS was analyzed by logistic regression under an "additive" model in which a numeric variable (0,1,2) is assigned based on the number of referent alleles. A class D regressive model was used to account for resemblance among siblings by including sample prevalence of young-onset diabetes among siblings as a covariate (15). Genomic control (16) was used to account for inflation of significance due to additional population stratification; the inflation parameter was calculated as the mean χ^2 statistic among all SNPs.

A family-based test of association among siblings discordant for diabetes was conducted by conditional logistic regression. To augment statistical power, the *P* value was calculated by combining the *P* value from conditional logistic regression (P_{sib}) with a truncated 1-sided *P* value from the case-control analysis (P_{CC1}). The family-based *P* value is thus taken from $\chi^2 = -2*\ln(P_{sib}) - 2*\ln(\max\{P_{sib}, [1-(1-P_{CC1})^2]\})$ on four df. This enhances power of the family-based test while maintaining robustness to population stratification (17).

Associations with diabetes in replication studies and in the pooled combined data were examined by logistic regression, fit by the generalized estimating equation procedure to account for dependence among siblings. Association with continuous variables was analyzed similarly with a linear "mixed" model. Values were logarithmically transformed, and the regression coefficient was exponentiated to obtain the effect estimate expressed a multiplier. The logarithms of homeostasis model assessment values were standardized within different insulin assays; effect sizes are presented in SD units. All *P* values presented are two-sided. To compare Pima results with those in Europeans, publicly available results were obtained from the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) consortium (1). Heterogeneity between Pimas and Europeans was analyzed by the Q-statistic.

Functional Studies

Overexpression and knockdown of *DNER* were assessed in murine pancreatic β-cells (NIT1). For overexpression,

cells were transfected with 2 μg of pCMV6 expression vector (OriGene, Rockville, MD) containing *DNER* insert or empty vector control using Lipofectamine LTX (Life Technologies, Carlsbad, CA). For knockdown studies, cells were transfected with 150 pmol small interfering RNA (siRNA) targeting *DNER* or negative control siRNA using Lipofectamine RNAiMAX. Cells were harvested after 48 h incubation, and total RNA was isolated using the RNeasy mini kit (Qiagen, Valencia, CA). Residual genomic DNA was eliminated by on-column DNAase digestion. First strand cDNA was synthesized using the Advantage RT-for-PCR kit (Clontech, Mountain View, CA). mRNA levels were quantified by real-time PCR using SYBR green on an ABI 7900HT-Fast RT-PCR System (Life Technologies). Relative mRNA level was taken as the ratio of experimental data to control. Data were averaged from 7–8 transfection experiments.

RESULTS

GWAS

Among the 453,654 SNPs, the genomic inflation factor was 1.13 for the case-control test and 1.02 for the family-based conditional logistic regression test. Results of the GWAS are shown in Fig. 1. There were 260 SNPs with $P < 0.0003$ in the family-based analysis and 242 SNPs with $P < 0.0005$ in the case-control analysis (P value thresholds were taken to select ~250 SNPs); three SNPs met both criteria. Among these 499 SNPs, 319 tags were selected for genotyping in replication studies (with $r^2 > 0.95$ taken as indicative of redundancy).

Replication Studies

Results for replication studies are shown in Supplementary Table 2. Among 311 SNPs genotyped in the first replication sample, 41 showed directionally consistent association with $P < 0.10$. Ninety-six of the 311 SNPs, constituting 26 SNPs with $P < 0.001$ in the combined GWAS and first replication set and 70 "candidate" SNPs, were also genotyped in the second replication sample. The 20 SNPs with the strongest associations in all 7,674 individuals are shown in Table 1. Two SNPs, rs8181588 in *KCNQ1* and rs1861612 in *DNER*, showed consistent and statistically strong ($P < 5 \times 10^{-7}$) evidence for association across all samples. The *KCNQ1* SNP tags rs2283228 (r^2 = 0.98), a previously established marker for T2DM (4). The *DNER* SNP has not been reported in other studies of T2DM, and the effect of rs1861612 is significantly different between Europeans and Pimas ($P = 1.6 \times 10^{-6}$; I^2 = 95.6%).

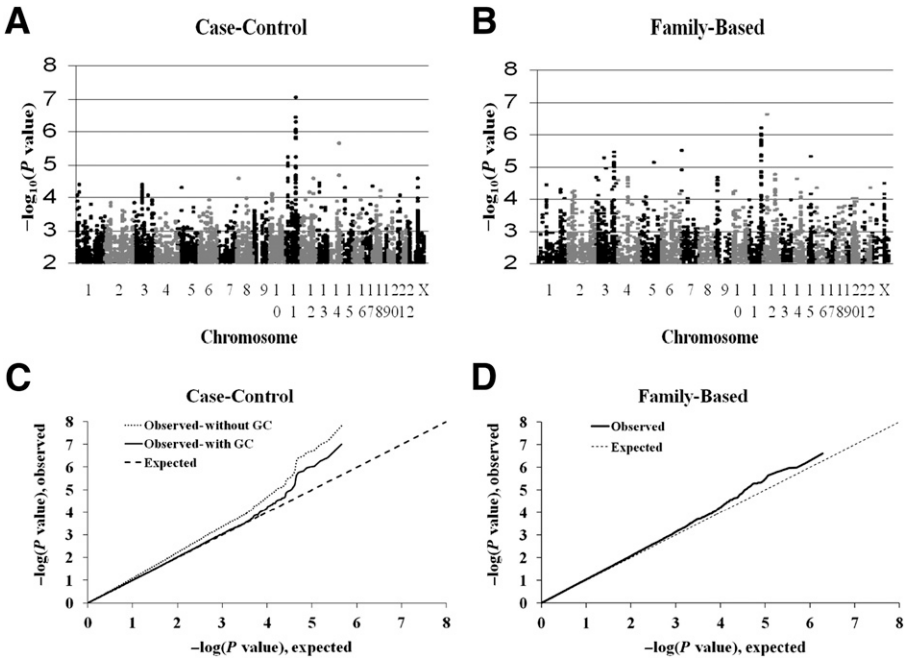

Figure 1—*A*: "Manhattan" plot of genome-wide association results for young-onset T2DM in American Indians in case-control study. The negative base 10 logarithm of the *P* value for an association with diabetes is plotted against chromosome and position (determined in Build 37). Results are shown after genomic control. *B*: "Manhattan" plot of genome-wide association results for young-onset T2DM in American Indians in family-based analysis. The negative base 10 logarithm of the *P* value for an association with diabetes is plotted against chromosome and position (determined in Build 37). *C*: Quantile–quantile plot of observed vs. expected (given the null distribution) *P* value for the case-control study. The observed distribution of *P* values without genomic control is shown in the dotted line, and the distribution with genomic control is shown in the solid line. *D*: Quantile–quantile plot of observed vs. expected (given the null distribution) *P* value for the family-based study. The expected distribution was estimated from simulation of 10^8 test statistics with the observed correlation (r = 0.29) between those for the conditional logistic regression and case-control analyses. GC, genomic control.

Table 1—Twenty SNPs with the strongest association with T2DM diabetes in all American Indian participants

						GWAS				Replication								
						Case-control		Family-based		Set 1		Set 2		All participants		Europeans		
SNP	Gene	Ch	Mb	Als	Freq	OR	*P* value	OR	*P* value	OR	*P* value	OR	*P* value	OR	*P* value	Freq	OR	*P* value
rs10909855	*ACTRT2*	1	2.91	T/C	0.38	1.76	1.4×10^{-4}	1.56	0.0095	1.12	0.1425	1.17	0.0282	1.19	1.2×10^{-4}	0.14	1.07	0.3013
rs10429895	*LOC440704*	1	190.66	T/C	0.75	1.54	0.0056	2.44	5.2×10^{-5}	1.18	0.0641	1.05	0.5537	1.19	4.7×10^{-4}	0.28	1.00	0.7695
rs10930939	*STAM2*	2	152.98	T/C	0.52	1.66	2.6×10^{-4}	1.12	0.6490	1.20	0.0124	1.06	0.4829	1.16	9.4×10^{-4}	0.25	1.00	0.7901
rs1861612	*DNER*	2	230.52	T/C	0.64	1.64	5.0×10^{-4}	1.19	0.5332	1.28	0.0016	1.28	0.0016	**1.29**	**6.6×10^{-8}**	0.57	0.99	0.6358
rs6774908	*C3orf58*	3	143.88	C/T	0.61	1.36	0.0290	2.13	2.1×10^{-4}	1.19	0.0256	1.12	0.1354	1.17	6.0×10^{-4}	0.56	1.00	0.7901
rs1861839	*MECOM*	3	169.26	A/G	0.57	1.65	3.6×10^{-4}	1.59	0.0027	1.13	0.1084	1.08	0.2635	1.16	0.0012	0.13	1.08	0.0743
rs7649407	*LPP*	3	188.15	A/G	0.88	2.40	3.1×10^{-4}	1.20	0.7060	1.31	0.0239	1.13	0.2355	1.28	4.8×10^{-4}	0.71	1.05	0.1448
rs33445	*ITGA2*	5	52.28	G/A	0.54	1.44	0.0105	1.91	1.4×10^{-4}	1.16	0.0305	1.08	0.3341	1.15	0.0018	0.43	0.96	0.1064
rs6901022	*KIF6*	6	39.31	A/C	0.61	1.47	7.2×10^{-4}	1.94	2.1×10^{-4}	1.11	0.1309	1.23	0.0056	1.18	2.7×10^{-4}	0.65	1.00	0.9680
rs4720763	*ICA1*	7	8.17	C/T	0.18	1.45	0.0294	3.40	1.4×10^{-5}	1.28	0.0154	1.09	0.3536	1.21	9.0×10^{-4}	0.55	1.03	0.4252
rs832234*	*PTPRD*	9	10.06	T/A	0.37	1.70	3.5×10^{-4}	1.19	0.4993	1.09	0.2808	1.16	0.0767	1.16	0.0020	0.15	—	—
rs590855	*MAP3K8*	10	30.76	C/T	0.16	2.08	2.4×10^{-4}	1.59	0.0487	1.07	0.4777	1.25	0.0331	1.24	1.8×10^{-4}	0.12	1.00	0.8766
rs11187815	*PLCE1*	10	95.97	G/A	0.28	1.50	0.0072	2.05	2.0×10^{-4}	1.26	0.0048	0.96	0.6038	1.17	0.0013	0.45	1.06	0.0991
rs8181588†	*KCNQ1*	11	2.83	A/G	0.48	1.36	0.0294	2.03	1.1×10^{-4}	1.25	0.0023	1.30	0.0003	**1.30**	**5.3×10^{-9}**	0.97	1.11	0.1165
rs4310643	*RAB38*	11	87.68	C/T	0.80	1.89	4.1×10^{-4}	1.45	0.0970	1.14	0.1310	1.09	0.3421	1.21	7.1×10^{-4}	0.85	0.99	0.7941
rs12296975	*FGD4*	12	32.71	G/A	0.41	1.54	0.0045	3.13	2.0×10^{-7}	1.05	0.4984	1.13	0.1054	1.15	0.0021	0.39	1.05	0.2873
rs659964‡	*ACAD10*	12	112.13	G/C	0.22	2.12	2.8×10^{-5}	1.23	0.4575	1.12	0.2336	1.18	0.0638	1.18	0.0019	0.20	0.90	0.0457
rs2434072	*TBX3*	12	114.99	T/C	0.42	1.63	4.6×10^{-4}	1.16	0.5772	1.14	0.0849	1.04	0.6227	1.15	0.0018	0.52	1.01	0.8167
rs523656	*STARD13*	13	33.72	A/G	0.45	1.78	3.9×10^{-5}	1.32	0.1322	1.09	0.2979	1.10	0.2186	1.18	5.0×10^{-4}	0.49	1.12	1.2×10^{-4}
rs17756627§	*RAD51B*	14	69.09	T/A	0.50	1.63	4.6×10^{-4}	1.39	0.7431	1.10	0.2285	1.08	0.2747	1.17	0.0003	0.19	1.02	0.5437

The nearest gene is listed, and the position (in Mb) is given from Build 37. Odds ratios are given per copy of this risk allele. Results for the GWAS analyses are adjusted for sex; results for replication sets 1 and 2 and all participants are adjusted for age, sex, birth year, and heritage. Associations with genome-wide statistical significance ($P < 5 \times 10^{-7}$ [10]) are shown in bold. Associations in Europeans, obtained from publicly available results from the DIAGRAM study (1) are shown for comparison; European allele frequencies are from the HapMap CEU population. CH, chromosome; Als, the alleles with the risk allele (in the GWAS case-control study) listed first; Freq, frequency of the risk allele; OR, odds ratio. *The *PTPRD* SNP rs832234 is not highly concordant with the established SNP rs17584499 ($r^2 = 0.01$ in Pimas). †Results for replication set 2 and all participants are given for rs2283228 ($r^2 = 0.98$ with rs8181588). ‡Results for *ACAD10* SNPs were reported previously (25). §Results for replication set 2 and all participants are given for rs4899250 ($r^2 = 0.96$ with rs17756627).

Table 2—Associations of GWAS-derived SNPs with quantitative metabolic constituents of T2DM

						BMI		HOMA-IR		HOMA-B		Percentage body fat		Insulin sensitivity (*M*)		Insulin secretion (AIR)	
SNP	Gene	Ch	Mb	Als	Freq	Eff	*P* value	Eff	*P* value	Eff	*P* value	Eff	*P* value	Eff	*P* value	Eff	*P* value
rs10909855	*ACTRT2*	1	2.91	T/C	0.38	0.998	0.7179	0.002	0.8755	−0.012	0.3924	1.022	0.2326	1.006	0.6991	0.909	0.0757
rs10429895	*LOC440704*	1	190.66	T/C	0.75	1.002	0.6076	**0.049**	**0.0026**	0.026	0.0786	1.019	0.3662	**0.963**	**0.0465**	0.954	0.4276
rs10930939	*STAM2*	2	152.98	T/C	0.52	**1.014**	**0.0008**	−0.008	0.6030	−0.002	0.8876	**1.040**	**0.0238**	0.995	0.7452	0.965	0.4811
rs1861612*	*DNER*	2	230.52	T/C	0.64	1.001	0.8413	**0.038**	**0.0119**	0.019	0.1474	1.011	0.5420	0.991	0.5872	1.075	0.1667
rs6774908	*C3orf58*	3	143.88	C/T	0.61	0.997	0.4736	−0.007	0.6309	−0.006	0.6260	0.990	0.6016	0.976	0.1437	0.976	0.6460
rs1861839	*MECOM*	3	169.26	A/G	0.57	1.003	0.4194	0.011	0.4692	−0.005	0.7065	0.986	0.4286	1.000	0.9951	1.027	0.6050
rs7649407	*LPP*	3	188.15	A/G	0.88	1.007	0.2499	−0.023	0.2964	**-0.042**	**0.0307**	1.008	0.7655	0.980	0.3862	1.002	0.9839
rs33445	*ITGA2*	5	52.28	G/A	0.54	1.002	0.7035	0.011	0.4349	−0.002	0.8966	0.996	0.8353	**0.969**	**0.0474**	0.993	0.8926
rs6901022	*KIF6*	6	39.31	A/C	0.61	1.008	0.0742	−0.005	0.7242	−0.005	0.6845	1.004	0.8219	0.998	0.8983	1.087	0.0961
rs4720763	*ICA1*	7	8.17	C/T	0.18	1.005	0.3854	0.034	0.0685	−0.003	0.8496	1.009	0.7128	0.967	0.1156	1.005	0.9369
rs832234	*PTPRD*	9	10.06	T/A	0.37	1.000	0.9723	0.002	0.8984	−0.000	0.9995	0.991	0.6133	1.011	0.5010	0.964	0.5217
rs590855	*MAP3K8*	10	30.76	C/T	0.16	1.002	0.7147	0.026	0.1965	0.025	0.1666	1.014	0.5838	0.990	0.6490	1.102	0.1899
rs11187815	*PLCE1*	10	95.97	G/A	0.28	1.002	0.6734	−0.004	0.8248	−0.010	0.4834	0.998	0.9068	0.980	0.2157	0.939	0.2214
rs2283228	*KCNQ1*	11	2.83	A/C	0.48	**0.983**	**4.0×10^{-5}**	−0.001	0.9671	−0.020	0.1406	0.977	0.1764	1.013	0.4053	**0.863**	**0.0038**
rs4310643	*RAB38*	11	87.68	C/T	0.80	1.008	0.1507	−0.007	0.7164	0.001	0.9483	0.996	0.8407	1.016	0.4226	**0.869**	**0.0217**
rs12296975	*FGD4*	12	32.71	G/A	0.41	0.996	0.3056	−0.022	0.1437	−0.011	0.4194	0.998	0.9147	0.996	0.8501	1.040	0.5052
rs659964	*ACAD10*	12	112.13	G/C	0.22	0.998	0.7034	−0.006	0.7457	−0.010	0.5252	0.986	0.5234	0.978	0.2463	1.134	0.0622
rs2434072	*TBX3*	12	114.99	T/C	0.42	1.008	0.0728	0.017	0.2519	0.003	0.8472	0.996	0.8120	0.981	0.2417	1.004	0.9422
rs523656	*STARD13*	13	33.72	A/G	0.45	1.006	0.1360	0.009	0.5615	0.011	0.4128	1.003	0.8698	0.982	0.3123	1.052	0.4045
rs4899250	*RAD51B*	14	69.0.09	T/C	0.49	1.006	0.1816	0.000	0.9782	−0.005	0.7090	1.005	0.7880	0.984	0.3188	1.005	0.9232

The nearest gene is listed, and the position (in Mb) is given from Build 37. The effect is given per copy of the risk allele. For BMI, percentage of body fat, insulin sensitivity, and insulin secretion the effect is expressed as a multiplier. For HOMA-IR and HOMA-B the effect is expressed as the difference in SD units per copy of the risk allele. Results for BMI are adjusted for age, sex, birth year, and heritage. Results for HOMA-IR and HOMA-B are adjusted for age, sex, birth year, heritage, and BMI. Results for percentage of body fat are adjusted for age, sex, and heritage. Results for *M* are adjusted for age, sex, heritage, and percentage of body fat. Results for acute insulin response are adjusted for age, sex, heritage, percentage of body fat, and *M*. Nominally significant results ($P < 0.05$) are shown in bold. Ch, chromosome; Als, the alleles with the diabetes risk allele listed first; Freq, frequency of the risk allele; Eff, effect; AIR, acute insulin response. *The association of rs1861612 with fasting serum insulin is effect = 0.040 SD; $P = 0.0158$.

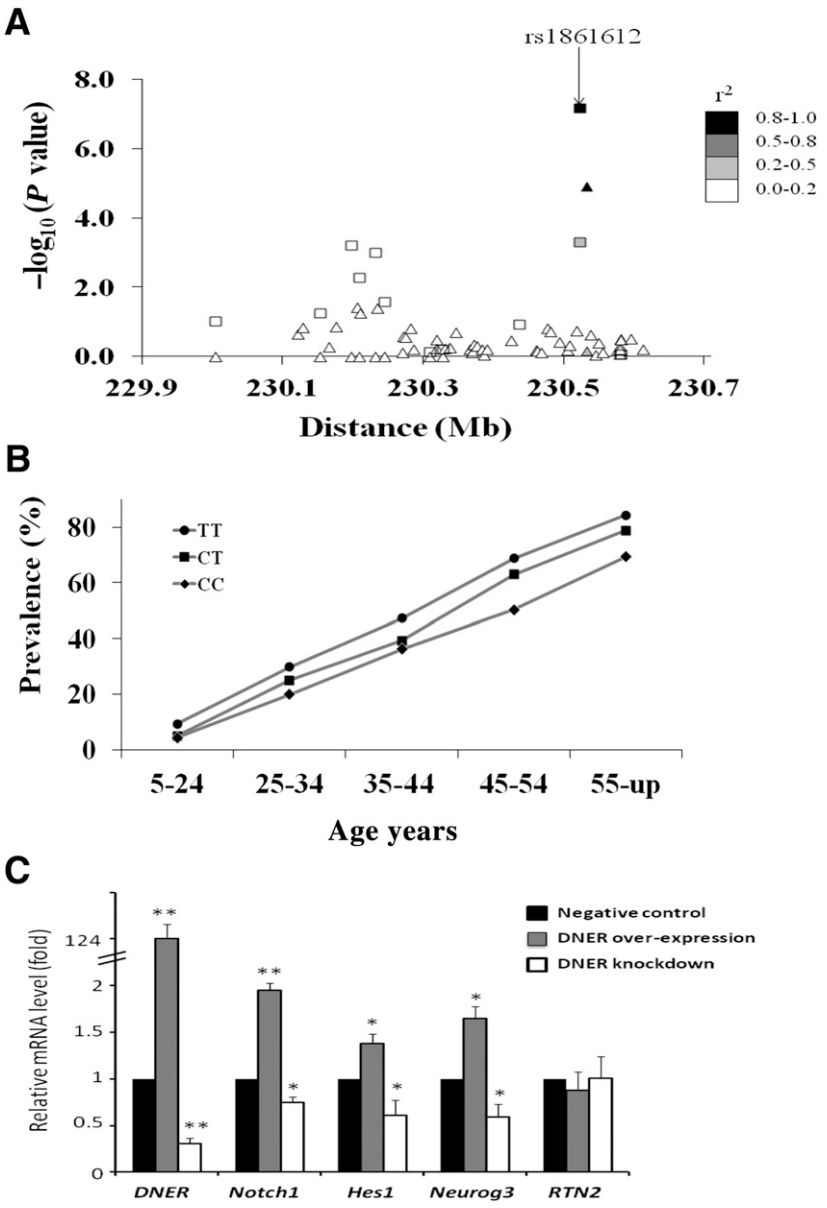

Figure 2—*A*: Association results for 63 variants across *DNER* with T2DM in Pima Indians. The negative base 10 logarithm of the *P* value for association is shown at the Build 37 position. Variants include 48 "tags" (r^2 >0.8) for 153 SNPs from the GWAS and 14 variants identified from sequencing ~5,300 base pairs in *DNER*; one variant identified by sequencing (a short insertion/deletion at position 230.580216 Mb) is not in public databases. Results obtained in all 7,674 individuals are shown as boxes, while those obtained in the GWAS and first replication samples are shown as triangles. Symbols are shaded according to r^2 with rs1861612. *B*: The prevalence of T2DM by genotype at rs1861612 and age-group in 7,674 Pima Indians. *P* values for association with T2DM in each age-group are as follows: *P* = 0.0005 (5–24), *P* = 0.0517 (25–34), *P* = 0.0265 (35–44), *P* = 0.0004 (45–54), and *P* = 0.0028 (55 and up). *C*: Relative mRNA level in murine pancreatic β-cells after transfection experiments to overexpress *DNER* (gray bars) or to knockdown *DNER* expression (open bars) compared with negative control (black bars, which = 1 by definition). mRNA levels are shown for *DNER* itself, for notch pathway genes (*Notch1*, *Hes1*, *Neurog3*) and for *Rtn2* (which is not involved in the notch pathway). **P* < 0.001; ***P* < 0.0001.

Associations With Metabolic Traits

Associations with metabolic traits are shown in Table 2. The diabetes risk allele at rs2283228 in *KCNQ1* was associated with lower insulin secretion (by 13.7% per copy of the risk allele; *P* = 0.0038). The diabetes risk allele at rs1861612 in *DNER* was associated with increased HOMA-IR and increased fasting serum insulin (~0.04 SD per copy of the risk allele; *P* = 0.0119).

Fine Mapping and Functional Studies

Sixty-three variants in *DNER* were selected from the GWAS and from direct sequencing of exons and ~2 kb of the promoter region in 12 diabetic and 12 nondiabetic Pimas. Results for association with diabetes are shown in Fig. 2*A*. The initial GWAS SNP, rs1861612, was the most strongly associated variant. The association was consistent across age-groups (Fig. 2*B*).

To investigate the role of *DNER* in regulating the notch signaling pathway, *DNER* mRNA was overexpressed (124-fold enrichment) and knocked down via siRNA (69% reduction) in murine β-cells, and expression levels of four genes were measured. The mRNA of notch pathway-specific genes *Notch1*, *Hes1*, and *Neurog3* were increased by 2.0-, 1.4-, and 1.6-fold, respectively ($P < 0.001$), in response to *DNER* overexpression and decreased by 25, 40, and 41% ($P < 0.001$) in response to *DNER* knockdown (Fig. 2*C*). In contrast, *Rtn2*, which is not involved in the notch pathway, was largely unaffected.

DISCUSSION

A number of genetic variants have recently been identified as associated with T2DM (1–6). Most of these variants were identified in GWASs in Europeans, but associations for many are consistent in other ethnic groups, including American Indians (18,19). However, some associations are heterogeneous across ethnic groups (5,6,20). In Pima Indians, for example, *TCF7L2* variants, which are strongly associated in most ethnic groups, show little association with diabetes (20). In addition because of ethnic differences in allele frequencies, relative importance of different diabetes-susceptibility alleles varies. For these reasons, GWASs in non-European populations might yield additional T2DM susceptibility variants. Indeed, studies in East Asians and South Asians have identified additional diabetes associations (4–6).

There have been few GWASs in American Indians despite their high risk for T2DM. In the current study, we extend our initial GWAS in Pima Indians (7) to include 453,654 SNPs. We also conduct additional replication studies in the population, so associations were ultimately assessed in 7,674 individuals. These studies implicate *DNER* as a novel locus conferring susceptibility to T2DM. The association with rs1861612 in *DNER* is reproducible across different groups of Pimas but is not observed in Europeans. Other ethnic-specific genetic associations for T2DM have been described (5,6). Such associations may occur due to difference in frequency of functional alleles, differences in linkage disequilibrium, or interaction with other genetic or environmental factors. Studies of *DNER* polymorphisms in other populations are required to determine generalizability.

Stringent criteria for statistical significance are generally applied in GWASs on account of multiple statistical testing. In non-African populations, $P < 5 \times 10^{-8}$ is conventionally considered genome-wide significance. In the current study, only a variant in the established diabetes gene *KCNQ1* achieved this threshold. Associations with *KCNQ1* variants, which are subject to parent-of-origin effects, are particularly strong in Pimas (21). Conventional criteria for genome-wide significance were derived from estimates of the number of effectively independent common variants in European and East Asian populations (22,23), and this number is likely smaller in Pimas. In fact, we estimate that $P < 5 \times 10^{-7}$ is an appropriate level for genome-wide statistical significance in Pimas (10), and the *DNER* association achieves this criterion. Although many of the other established T2DM-susceptibility loci appear to influence diabetes risk in Pimas (19), their effects are modest and difficult to detect at genome-wide significance with the current sample. The present GWAS was small and had little power in itself to detect associations at genome-wide significance.

The mechanism by which variation in *DNER* might cause T2DM is not clear. *DNER* encodes a Δ/notch-like epidermal-growth-factor-related receptor, and it mediates notch signaling. We show that *DNER* expression affects expression of several notch pathway genes in pancreatic β-cells. The diabetes risk allele at rs1861612 was associated with fasting hyperinsulinemia and elevated HOMA-IR, but there was no association with directly measured insulin resistance. A speculative mechanism is that *DNER*-mediated alterations in notch signaling may produce fasting hyperinsulinemia, which increases risk of diabetes independently of insulin resistance (24), but further mechanistic studies are required. Regardless of the mechanism, the current study implicates *DNER* as a T2DM-susceptibility gene in American Indians.

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