Robert L. Hanson, Rong Rong, Sayuko Kobes, Yunhua Li Muller, E. Jennifer Weil, Jeffrey M. Curtis, Robert G. Nelson, and Leslie J. Baier

Role of Established Type 2 Diabetes–Susceptibility Genetic Variants in a High Prevalence American Indian Population

Diabetes 2015;64:2646–2657 | DOI: 10.2337/db14-1715

Several single nucleotide polymorphisms (SNPs) associated with type 2 diabetes mellitus (T2DM) have been identified, but there is little information on their role in populations at high risk for T2DM. We genotyped SNPs at 63 T2DM loci in 3,421 individuals from a high-risk American Indian population. Nominally significant ($P < 0.05$) associations were observed at nine SNPs in a direction consistent with the established association. A genetic risk score derived from all loci was strongly associated with T2DM (odds ratio 1.05 per risk allele, $P = 6.2 \times 10^{-6}$) and, in 292 nondiabetic individuals, with lower insulin secretion (by 4% per copy, $P = 4.1 \times 10^{-6}$). Genetic distances between American Indians and HapMap populations at T2DM markers did not differ significantly from genomic expectations. Analysis of U.S. national survey data suggested that 66% of the difference in T2DM prevalence between African Americans and European Americans, but none of the difference between American Indians and European Americans, was attributable to allele frequency differences at these loci. These analyses suggest that, in general, established T2DM loci influence T2DM in American Indians and that risk is mediated in part through an effect on insulin secretion. However, differences in allele frequencies do not account for the high population prevalence of T2DM.

In recent years, more than 70 distinct genomic regions have been identified in which single nucleotide polymorphism (SNP) markers show reproducible association with type 2 diabetes mellitus (T2DM) at genome-wide statistical significance ($P < 5 \times 10^{-8}$) (1–17). Most of these variants were discovered by genome-wide association studies (GWAS) in European populations, and their effects are best characterized in populations of European ancestry. Studies in other ethnic groups suggest that effects on T2DM are similar to those seen in Europeans for most variants (18,19), but clear examples of heterogeneity in effects have been observed (8,10,20). There is limited information on the role of these established variants in populations at high risk for T2DM or on the extent to which differences in allele frequencies at these variants account for differences in population risk. In the present study, we analyze 63 established T2DM-susceptibility variants in Pima Indians, an American Indian population in whom the prevalence of T2DM is extraordinarily high (21).

RESEARCH DESIGN AND METHODS

Participants

Subjects were participants in a longitudinal study conducted in the Gila River Indian Community in central Arizona, where most residents are Pima Indians (21). The present study consisted of 3,421 individuals whose self-reported heritage was full Pima, Tohono O'odham, or a mixture of these closely related tribes and who had DNA available. These individuals constituted 1,951 sibships. There were 1,964 women and 1,457 men; mean ± SD age at last examination was 40.6 ± 16.5 years. Height and weight were measured, and a 75-g oral glucose tolerance test was administered; diabetes was diagnosed in 1,615 individuals (47.2%) according to 1997 American Diabetes Association criteria (22), i.e., 2-h postload plasma glucose ≥11.1 mmol/L, fasting plasma glucose ≥7.0 mmol/L, or a diagnosis during routine clinical care.

Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Phoenix, AZ

Corresponding author: Robert L. Hanson, rhanson@phx.niddk.nih.gov.

Received 7 November 2014 and accepted 3 February 2015.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db14-1715/-/DC1.

A subset of individuals participated in detailed physiologic studies to assess metabolic predictors of T2DM. Body composition was measured by hydrodensitometry or by DEXA, as previously described (23), in 405 nondiabetic full-heritage Pimas (172 women and 233 men; mean ± SD age 26.7 ± 6.1 years). Insulin sensitivity was measured in these 405 individuals by the hyperinsulinemic-euglycemic clamp (23). Insulin was infused at physiologic levels (~130 μmol/L), and glucose was infused to maintain euglycemia. Rate of glucose uptake, normalized to estimated metabolic body size (EMBS), was taken as a measure of insulin sensitivity (*M*) (milligrams per kilogram EMBS per minute). Insulin secretion was measured as the acute insulin response (microunits per milliliter) 3–5 min after a 25-g intravenous glucose challenge (23) in 292 individuals (105 women and 187 men; mean ± SD age 26.7 ± 6.1 years) with normal glucose tolerance (2-hour postload glucose <7.8 mmol/L).

Genotyping

A sentinel SNP for each region was selected for genotyping from previously reported GWAS (1–17). Two SNPs were selected for *KCNQ1* and *CDC123*, where two distinct sets of variants have been described. In addition, 45 ancestry-informative markers (24) were genotyped for estimation of the individual proportion of European heritage (25). Genotyping was conducted by the SNPplex method (Life Technologies, Carlsbad, CA) or the BeadXpress system (Illumina, San Diego, CA) according to the manufacturer's instructions. Results for 18 SNPs were reported previously (20,26–29). They are included here for a more complete characterization of the effects of established T2DM loci.

Association with T2DM and Related Traits

Association between genotype and T2DM at the last research examination was analyzed by a logistic regression model, which was fit by the generalized estimating equation procedure to account for sibship. Genotype was coded as a numeric variable representing number of risk alleles as defined in previous GWAS. Thus, an odds ratio (OR) >1 indicates association in the same direction as the established association and an OR <1 indicates association in the opposite direction. Continuous variables were analyzed with a linear mixed model in which genotype and other covariates were fixed effects and sibship was a random effect. The logarithm of each variable was analyzed, and the regression coefficient was exponentiated to obtain the effect per copy of the T2DM risk allele, expressed as a multiplier.

For assessment of whether associations in Pimas were consistent with those in Europeans, ORs were compared by the Cochran Q test of homogeneity, and heterogeneity was quantified by the I^2 measure (30). ORs for Europeans were taken from previous publications (1,2,8,9,14–17,31–40). For assessment of whether GWAS-defined risk alleles contribute in aggregate to T2DM in Pimas, a multiallelic genetic risk score (GRS) was created by summing the number of risk alleles over all loci. To avoid reduction in sample size resulting from missing data at a few loci, we calculated the probability that an individual was of each possible genotype for each missing value from the genotypes in the individual's relatives using MLINK (41); these probabilities were used in calculating the GRS.

To test for heterogeneity across all loci, we combined *P* values derived from the heterogeneity test for individual SNPs by constructing a signed *Z* score. The *Z* score was computed for each SNP as $Z_i = \text{sign}[\ln(OR_{EUi}) - \ln(OR_{PIi})]\Phi^{-1}(P_{heti}/2)$, where OR_{EUi} represents the OR for the ith SNP in Europeans, OR_{PIi} represents the corresponding OR in Pimas, P_{heti} is the *P* value for heterogeneity, and Φ^{-1} represents the inverse of the cumulative normal probability function. The sum of the *Z* scores across all SNPs divided by the square root of the number of SNPs (Z^*) was used to calculate a *P* value for the null hypothesis of homogeneity across all markers (42). If Z^* is negative, it indicates that ORs on average are weaker in Pimas than in Europeans, whereas if Z^* is positive it indicates that ORs are stronger in Pimas.

Differences in Allele Frequencies

Frequency of the risk allele was estimated by maximum likelihood methods using the ILINK program to account for family membership (41). Data for these 63 SNPs were obtained from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) or, if not available from HapMap, from the 1000 Genomes Project (http://www.1000genomes.org/). For comparison of allele frequencies with other major continental ethnic groups, data were obtained for individuals of European ancestry from Centre d'Etude du Polymorphisme Humain families in Utah (CEU), for East Asians from Han Chinese in Beijing (CHB), and for Africans from Yoruba in Ibadan, Nigeria (YRI), HapMap populations. The likelihood ratio test was used to test significance of the difference in risk allele frequency in Pimas ($f_{R\text{-}Pima}$) with that in each HapMap population ($f_{R\text{-}CEU}$, $f_{R\text{-}CHB}$, and $f_{R\text{-}YRI}$). For assessment of whether T2DM risk alleles were systematically higher in one population than in another, the mean of the GRS (μ_{GRS}) was compared between populations.

For a more general comparison of genetic distance between Pimas and other populations, the coancestry coefficient (F_{ST}) was calculated across all T2DM-susceptibility variants by the method of moments (43). Since interpretation of F_{ST} is most straightforward when sample sizes are equal, Pima allele frequencies used in these calculations were derived from a random sample of equal effective size to the corresponding HapMap population; effective sample size was estimated by the method of Yang et al. (44). For comparison of F_{ST} calculated across the T2DM markers with its genomic expectation, random markers were selected from a GWAS in Pimas (45). Since SNP characteristics may have influenced detection of the T2DM markers, each T2DM-associated SNP was matched to potential random SNPs by minor allele frequency in CEU, base pair type, and chromosome type (autosomal vs. X chromosome); to avoid selecting markers highly

concordant with those for susceptibility to T2DM, we excluded a 2-Mb region on either side of the sentinel SNP from this selection. A total of 294,467 potentially matching random SNPs were thus identified. Significance of the difference between F_{ST} at T2DM variants and F_{ST} at random markers was calculated by a bootstrap procedure in which one random marker was selected for each T2DM variant in each iteration.

Excess Population Prevalence Attributable to Allele Frequency Differences

For quantification of the extent to which differences in T2DM risk allele frequencies can explain the difference in T2DM prevalence between Pimas and Europeans, standard multivariable epidemiologic methods for calculation of attributable fraction (46) were modified to calculate the genetic attributable fraction (GAF) for the population difference in prevalence. We define this as the proportion of the excess T2DM prevalence in a high-risk "target" population compared with a low-risk "reference" population attributable to differences in risk allele frequency. If P_0 represents prevalence in the reference population (e.g., Europeans) and P_1 is prevalence in the target population (e.g., Pimas), then

$$\mathrm{GAF} = 1 - \left(P_{1\mathrm{adj}} - P_0\right)/\left(P_1 - P_0\right)$$

where $P_{1\mathrm{adj}}$ is prevalence in the target population adjusted for the allele frequency differences (i.e., prevalence if the target population had the same risk allele frequencies as the reference population) (Eq. 1). Data from non-Hispanic white participants in the oral glucose tolerance subset of the U.S. National Health and Nutrition Examination Survey (NHANES) 2005–2010 were used for the reference population (http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm). These data were from 3,282 individuals age 12–84 years (1,585 women, 1,697 men; mean ± SD age 46.8 ± 20.1 years); 523 individuals (15.9%) had diabetes. These data were combined with Pimas of the same age range for calculation of GAF.

If genotypic data for all markers were available for all individuals, the quantities needed to calculate GAF could be derived from multivariable logistic regression. However, such data are not readily available for NHANES participants, so we developed an approximation that uses allele frequency and OR estimates from other sources. Adjusted prevalence in each population was obtained from the following logistic regression equation: logit (prevalence) = $\alpha_0 + \alpha_1 I + \gamma_1(\mathrm{cov}_1) + \ldots \gamma_m(\mathrm{cov}_m)$, where I is an indicator variable that takes the value of 0 for the reference population and 1 for the target population and γ_1–γ_m represent the coefficients corresponding to m covariates (centered at the mean values in the target population). Under an additive model with assumptions of Hardy-Weinberg equilibrium in both populations, independence among SNPs, and that the population OR changes as a function of the OR associated with each SNP and the difference in risk allele frequency, the expected value of α_1, given that allele frequencies are the same as in the reference population, is as follows: $\alpha_{1\mathrm{adj}} = \alpha_1 - \Sigma[2\beta_i(f_{R1i}{}^2 + f_{R1i}[1 - f_{R1i}] - f_{R0i}{}^2 - f_{Roi}[1 - f_{Roi}])]$, where β_i is the logarithm of the OR for the ith SNP, f_{R1i} is the risk allele frequency in the target population, and f_{R0i} is the frequency in the reference population. For the present analyses, allele frequencies in the HapMap CEU population were taken as representative of the reference population. The values required for calculation of GAF (see Eq. 1) are as follows:

$$P_0 = 1\Big/\left(1 + e^{-\alpha 0}\right),$$
$$P_1 = 1\Big/\left(1 + e^{-(\alpha 0 + \alpha 1)}\right),\ P_{1\mathrm{adj}} = 1\Big/\left(1 + e^{-(\alpha 0 + \alpha 1\mathrm{adj})}\right)$$

Simulation studies suggest that estimates of GAF derived by this method provide a good approximation of those derived from a multivariable regression in which all data are available for all individuals (Fig. 1). CIs and hypothesis tests for GAF were derived from a bootstrap procedure in which Pima, NHANES, and CEU populations were resampled and ORs were sampled from published values and standard errors.

RESULTS

Association with T2DM

Eight SNPs (rs17106184 in *FAF1*, rs7578597 in *THADA*, rs3923113 in *GRB14*, rs831571 in *PSMD6*, rs6808574 in *LPP*, rs1531343 in *HMGA2*, rs7957197 in *HNF1A*, and rs17782313 in *MC4R*) were nearly monomorphic (minor allele frequency <0.01) in Pimas and were not analyzed for association. Table 1 shows the association for each of the remaining 55 SNPs with T2DM in Pima Indians, along with the test for heterogeneity in ORs between Pimas and Europeans. Nine SNPs, those in *GCKR, ZBED3, CDKAL1, ZFAND3, KCNQ1, SPRY2, HMG20A, PRC1*, and *FTO*, had nominally significant associations ($P < 0.05$) in Pimas in the same direction as the established association. The previously reported result with the *KCNQ1* SNP rs2237892 was the strongest association. Ten SNPs, in *IRS1, ADAMTS9, ARL15, ZFAND3, PTPRD, TCF7L2, MPHOSPH9, C2CD4A, SLC16A11*, and *DUSP9*, showed nominally significant heterogeneity between Pimas and Europeans. Nonetheless, ORs were in the same direction as the established association for 39 of the 55 SNPs.

Associations with Metabolic Predictors of T2DM

Results for SNPs with nominally significant and directionally consistent associations with metabolic traits are shown in Table 2. Results for all SNPs are shown in Supplementary Table 1. The T2DM risk allele was associated with lower insulin secretion for SNPs in *PROX1, IGF2BP2, ZBED3, DGKB-TMEM195, GLIS3, CDC123, HHEX, KCNQ1*, and *MNTR1B*. The T2DM risk allele for SNPs in *IRS1, PPARG, MNTR1B, PRC1*, and *SRR* was associated with lower values of insulin sensitivity. Since the *MNTR1B* SNP was associated with both insulin

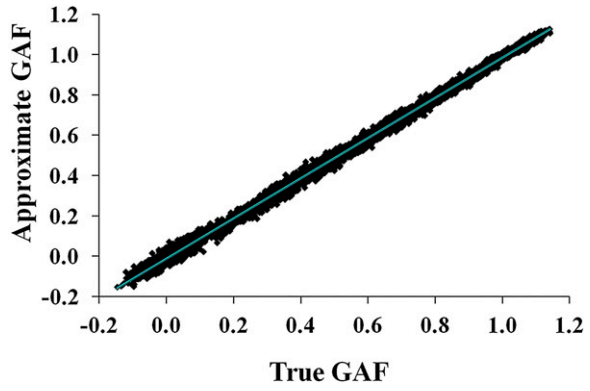

Figure 1—Simulations comparing the approximate method for estimation of GAF with the "true" GAF, estimated from a multivariable logistic regression model containing all SNPs. Simulations were conducted generating 100,000 individuals from a reference population and 3,000 individuals from a target population. Twenty-five pairs of susceptibility loci were generated from a distribution with allele frequency differences between target and reference populations given F_{ST} = 0.15 such that each locus was matched to another with the same minor allele frequency in each population. Phenotypes were generated with each locus contributing ~0.15% to disease liability, along with a residual population effect such that the OR comparing the target with reference population was ~10. The expected GAF was controlled by specifying the proportion of matched pairs for which the risk allele had a higher frequency in the target population for both members of the pair (e.g., if for each matched pair of loci one has a higher frequency in the target population and the other has a higher frequency in the reference population, the expected GAF is ~0, while if all loci have higher risk allele frequency in the reference population, expected GAF is ~1). The true GAF was calculated from logistic regression models with all 103,000 individuals. For simulation of the situation in the present study, for the approximate method, allele frequencies for the reference population were calculated from a subset of 180 individuals and the prevalence estimates between populations from a subset of 3,000 individuals from the reference population. Six thousand replicate data sets were analyzed. Owing to chance variation in the simulation, both true and approximate GAF may be <0 or >1.

secretion and total body insulin sensitivity, we further investigated its relationship with hepatic insulin sensitivity, measured in the clamp using radiolabeled glucose, and found that the risk allele was associated with lower sensitivity ($r = -0.15$, $P = 0.002$). The T2DM risk allele was significantly associated with higher percentage body fat for SNPs in *PRC1* and *ZFAND3*. When BMI was analyzed in the larger population, the T2DM risk alleles for variants in *GCK* and *FTO* were associated with significantly higher BMI (Supplemental Table 2).

Multiallelic Association

Associations with the multiallelic GRS are shown in Fig. 2. The sum of the number of risk alleles over all 55 SNPs was significantly associated with T2DM (OR 1.05 per copy of a risk allele, $P = 6.2 \times 10^{-6}$). There was also a strong association between a greater number of T2DM risk alleles and lower values of insulin secretion such that each copy of a risk allele was associated with a 4% decrease in insulin secretion ($P = 4.1 \times 10^{-6}$). There was little association with insulin sensitivity or percentage body fat. When alleles were weighted by the logarithms of the published ORs in constructing the GRS, similar results were obtained (data not shown). When BMI was analyzed, results were similar to those seen with percentage body fat, but the inverse association was statistically significant (lower by 0.4% per risk allele, $P = 1.2 \times 10^{-5}$) (Supplementary Fig. 1). When the GRS was constructed using only the nine insulin secretion–associated SNPs, each risk allele was associated with a 13% decrease in insulin secretion; similarly, in a score constructed from the five insulin sensitivity SNPs, each risk allele was associated with a 7% decrease in insulin sensitivity (Supplementary Fig. 2). The insulin secretion score was associated with T2DM (OR 1.09, $P = 2.7 \times 10^{-4}$); when these nine SNPs were excluded from the global GRS, the T2DM association was modestly attenuated (OR 1.04, $P = 1.1 \times 10^{-3}$).

Heterogeneity

The test for heterogeneity in the effect on T2DM across all SNPs was statistically significant ($P = 3.9 \times 10^{-5}$) and negative in sign ($Z^* = -4.12$); this indicates that the effects of these SNPs are on average weaker in Pimas than in Europeans. When the 18 SNPs with nominally significant association with T2DM or significant heterogeneity were excluded, the effects of the GRS on T2DM (OR 1.04, $P = 4.9 \times 10^{-4}$) and insulin secretion (effect −4%, $P = 8.0 \times 10^{-6}$) remained significant, as did evidence for heterogeneity ($Z^* = -2.82$, $P = 4.8 \times 10^{-3}$).

Allele Frequency Differences between Pimas and Major Continental Populations

The difference in frequency of the T2DM risk allele between Pimas and HapMap populations is shown for each locus in Supplementary Fig. 3. The distribution of the GRS in each population is shown in Fig. 3. Mean GRS in Pimas (68.4) was slightly but significantly lower than in CEU (69.2, $P = 0.049$); mean GRS in Pimas was also significantly lower than in YRI (73.7, $P = 4.4 \times 10^{-38}$) but higher than in CHB (66.6, $P = 9.6 \times 10^{-5}$). When loci were weighted by the logarithms of the ORs in constructing the GRS, results were similar, except that the contrast in mean GRS between Pimas and CEU was more pronounced ($P = 1.2 \times 10^{-10}$).

Genetic distances among populations across all 63 T2DM markers and across random markers are summarized in Fig. 4. F_{ST} across these T2DM loci was 0.163 (95% CI 0.154, 0.173) between Pimas and CEU, 0.138 (0.125, 0.152) between Pimas and CHB, and 0.232 (0.221, 0.244) between Pimas and YRI. These values were not significantly different from those derived from matched sets of SNPs randomly selected across the genome: F_{ST} 0.158 (0.106, 0.209) between Pimas and CEU ($P = 0.83$ for difference in F_{ST}), 0.129 (0.078, 0.180) between Pimas and CHB ($P = 0.74$), and 0.241 (0.173, 0.309) between Pimas and YRI ($P = 0.80$). Thus, differences in allele frequency are generally similar to those expected given genetic distances between populations.

Table 1—Association of variants with T2DM in Pima Indians and comparison with association in Europeans

				Proportion of Pimas with Diabetes (*n*)			Pima Indians		Europeans		Heterogeneity		
Gene	SNP	Allele R/L	Pima freq.	RR	LR	LL	*P*	OR (95% CI)	Freq.	OR (95% CI)	I^2	P_{het}	Ref.
NOTCH2	rs10923931	T/G	0.10	0.37 (27)	0.46 (540)	0.47 (2,622)	0.5803	0.94 (0.76–1.17)	0.09	1.13 (1.09–1.17)	63	0.0980	1
PROX1	rs340874	C/T	0.35	0.49 (353)	0.47 (1,488)	0.46 (1,388)	0.4563	1.05 (0.93–1.19)	0.57	1.07 (1.05–1.09)	0	0.7444	14
GCKR	rs780094	C/T	0.95	0.47 (2,982)	0.40 (278)	0.32 (7)	**0.0230**	**1.38 (1.04–1.81)**	0.59	1.06 (1.04–1.08)	71	0.0638	14
BCL11A	rs243021	A/G	0.78	0.48 (1,987)	0.46 (1,109)	0.41 (151)	0.1775	1.10 (0.96–1.28)	0.47	1.08 (1.06–1.10)	0	0.7630	2
IRS1	rs7578326	A/G	0.91	0.45 (2,724)	0.54 (517)	0.44 (27)	**0.0081**	**0.76 (0.62–0.93)**	0.65	1.11 (1.09–1.13)	**92**	**0.0003**	2
PPARG	rs1801282	C/G	0.92	0.47 (2,758)	0.45 (477)	0.37 (19)	0.3807	1.10 (0.89–1.37)	0.90	1.16 (1.09–1.23)	0	0.6649	31
UBE2E2	rs7612463	C/A	0.97	0.46 (3,073)	0.55 (191)	0.00 (4)	0.1080	0.76 (0.55–1.06)	0.87	1.05 (0.97–1.14)	71	0.0656	1
ADAMTS9	rs4607103	C/T	0.62	0.45 (1,102)	0.47 (1,432)	0.49 (418)	0.2405	0.93 (0.81–1.05)	0.81	1.09 (1.06–1.12)	**83**	**0.0155**	1
ADCY5	rs11708067	A/G	0.50	0.47 (821)	0.47 (1,594)	0.46 (807)	0.6991	1.02 (0.90–1.16)	0.78	1.12 (1.09–1.15)	47	0.1715	14
IGF2BP2	rs4402960	T/G	0.17	0.52 (99)	0.46 (853)	0.47 (2,166)	0.6191	1.04 (0.89–1.21)	0.30	1.13 (1.10–1.16)	8	0.2976	32
ST6GAL	rs16861329	C/T	0.44	0.46 (562)	0.48 (1,576)	0.45 (979)	0.5209	1.04 (0.92–1.18)	0.88	1.02 (0.95–1.09)	0	0.7760	9
MAEA	rs6815464	C/G	0.61	0.48 (1,278)	0.46 (1,442)	0.46 (470)	0.4159	1.05 (0.93–1.19)	0.99	1.19 (1.04–1.36)	45	0.1761	8
WFS1	rs10010131	G/A	0.98	0.47 (3,019)	0.36 (92)	0.00 (1)	0.0590	1.59 (0.98–2.58)	0.67	1.12 (1.08–1.16)	51	0.1544	33
TMEM154	rs6813195	C/T	0.51	0.48 (850)	0.47 (1,635)	0.44 (800)	0.1850	1.08 (0.96–1.21)	0.72	1.08 (1.05–1.11)	0	0.9851	17
ALR15	rs702634	A/G	0.99	0.46 (3,221)	0.63 (77)	— (0)	**0.0351**	**0.51 (0.27–0.95)**	0.71	1.05 (1.02–1.08)	**80**	**0.0240**	17
ZBED3	rs4457053	G/A	0.36	0.52 (441)	0.47 (1,481)	0.44 (1,346)	**0.0246**	**1.15 (1.02–1.31)**	0.26	1.08 (1.05–1.11)	3	0.3107	2
SSR1	rs9505118	A/G	0.53	0.50 (877)	0.46 (1,495)	0.44 (686)	0.0621	1.12 (0.99–1.27)	0.62	1.06 (1.03–1.09)	0	0.3651	17
CDKAL1	rs7756992	G/A	0.32	0.52 (372)	0.47 (1,352)	0.45 (1,448)	**0.0366**	**1.15 (1.01–1.30)**	0.28	1.22 (1.17–1.27)	0	0.3548	34
POU5F1	rs3130501	G/A	0.61	0.47 (1,180)	0.47 (1,570)	0.45 (536)	0.5962	1.03 (0.92–1.16)	0.73	1.06 (1.02–1.10)	0	0.6720	17
ZFAND3	rs9470794	C/T	0.03	0.82 (3)	0.56 (179)	0.46 (3,038)	**0.0069**	**1.56 (1.13–2.16)**	0.12	0.98 (0.88–1.09)	**86**	**0.0073**	1
DGKB-TMEM195	rs2191349	T/G	0.19	0.47 (117)	0.46 (1,017)	0.47 (2,116)	0.9503	1.00 (0.86–1.16)	0.47	1.06 (1.04–1.08)	0	0.4150	14
JAZF1	rs864745	T/C	0.78	0.48 (1,947)	0.44 (1,003)	0.44 (137)	0.1250	1.13 (0.97–1.31)	0.50	1.10 (1.07–1.13)	0	0.7708	1
GCK	rs4607517	A/G	0.34	0.46 (359)	0.47 (1,504)	0.47 (1,359)	0.8376	0.99 (0.87–1.12)	0.19	1.07 (1.04–1.10)	36	0.2126	14
GCC1-PAX4	rs6467136	G/A	0.29	0.46 (300)	0.47 (1,315)	0.47 (1,629)	0.7316	0.98 (0.86–1.11)	0.50	1.00 (0.94–1.06)	0	0.7551	1
KLF14	rs972283	G/A	0.38	0.52 (442)	0.45 (1,415)	0.46 (1,121)	0.1854	1.09 (0.96 –1.24)	0.55	1.07 (1.04–1.10)	0	0.7865	2
TP53INP1	rs896854	T/C	0.48	0.48 (736)	0.47 (1,631)	0.45 (879)	0.2439	1.07 (0.95–1.21)	0.43	1.06 (1.03–1.09)	0	0.8455	2
SLC30A8	rs13266634	C/T	0.91	0.47 (2,658)	0.45 (520)	0.53 (33)	0.6870	1.04 (0.85–1.29)	0.76	1.14 (1.12–1.16)	0	0.4121	35
GLIS3	rs7041847	A/G	0.66	0.48 (1,334)	0.45 (1,468)	0.46 (377)	0.2742	1.07 (0.95–1.22)	0.55	1.04 (0.98–1.10)	0	0.6599	1
PTPRD	rs17584499	T/C	0.10	0.29 (38)	0.42 (537)	0.48 (2,528)	**0.0081**	**0.75 (0.60–0.93)**	0.23	1.03 (0.94–1.13)	**86**	**0.0074**	1
CDKN2B	rs10811661	T/C	0.94	0.47 (2,830)	0.44 (362)	0.50 (10)	0.3518	1.13 (0.87–1.46)	0.80	1.22 (1.17–1.27)	0	0.5664	36
CHCHD9	rs13292136	C/T	0.66	0.47 (1,445)	0.47 (1,419)	0.43 (400)	0.4280	1.05 (0.93–1.19)	0.93	1.11 (1.07–1.15)	0	0.3891	2

Continued on p. 2651

Table 1—Continued

Gene	SNP	Allele R/L	Pima freq.	Proportion of Pimas with Diabetes (*n*)			Pima Indians		Europeans		Heterogeneity		Ref.
				RR	LR	LL	*P*	OR (95% CI)	Freq.	OR (95% CI)	I^2	P_{het}	
CDC123	rs10906115	A/G	0.57	0.46 (1,015)	0.47 (1,504)	0.48 (605)	0.5695	0.96 (0.85–1.09)	0.64	1.07 (1.00–1.14)	53	0.1433	1
CDC123	rs12779790	G/A	0.15	0.53 (89)	0.45 (711)	0.47 (2,260)	0.9526	1.00 (0.85–1.17)	0.23	1.11 (1.08–1.14)	41	0.1935	1
VPS26A	rs1802295	T/C	0.25	0.43 (170)	0.48 (1,126)	0.46 (1,667)	0.8955	1.01 (0.87–1.17)	0.35	1.04 (0.99–1.09)	0	0.7076	9
HHEX	rs1111875	C/T	0.39	0.49 (467)	0.47 (1,540)	0.45 (1,176)	0.2025	1.08 (0.96–1.22)	0.58	1.16 (1.09–1.23)	2	0.3130	37
TCF7L2	rs7903146	T/C	0.08	0.53 (27)	0.47 (397)	0.46 (2,590)	0.6314	1.06 (0.84–1.35)	0.29	1.38 (1.32–1.44)	**78**	**0.0327**	38
KCNQ1	rs231362	G/A	0.93	0.47 (2,673)	0.43 (332)	0.49 (20)	0.2890	1.15 (0.89–1.47)	0.51	1.08 (1.06–1.10)	0	0.6453	2
KCNQ1	rs2237892	C/T	0.51	0.52 (823)	0.48 (1,643)	0.39 (803)	**9.4×10^{-6}**	**1.31 (1.16–1.48)**	0.92	1.19 (1.11–1.28)	47	0.1713	39
KCNJ11	rs5219	T/C	0.38	0.48 (505)	0.47 (1,583)	0.45 (1,252)	0.3823	1.05 (0.94–1.19)	0.37	1.12 (1.08–1.16)	0	0.3386	40
CENTD2	rs1552224	A/C	0.95	0.47 (2,987)	0.39 (284)	0.89 (3)	0.0606	1.33 (0.99–1.78)	0.87	1.14 (1.11–1.17)	0	0.3167	2
MNTR1B	rs1387153	T/C	0.11	0.44 (46)	0.47 (635)	0.47 (2,663)	0.9000	1.01 (0.84–1.22)	0.27	1.12 (1.07–1.17)	4	0.3084	2
TSPAN8	rs7961581	C/T	0.01	— (0)	0.31 (60)	0.47 (2,999)	0.0907	0.51 (0.24–1.11)	0.26	1.09 (1.06–1.12)	73	0.0563	1
MPHOSPH9	rs4275659	C/T	0.44	0.47 (622)	0.45 (1,651)	0.50 (1,031)	0.2839	0.94 (0.83–1.06)	0.67	1.06 (1.03–1.09)	**74**	**0.0497**	17
SPRY2	rs1359790	G/A	0.64	0.48 (1,271)	0.48 (1,431)	0.39 (425)	**0.0408**	**1.14 (1.01–1.29)**	0.73	1.11 (1.04–1.18)	0	0.7115	1
C2CD4A	rs7172432	A/G	0.37	0.39 (428)	0.47 (1,423)	0.48 (1,176)	**0.0252**	**0.86 (0.76–0.98)**	0.57	1.07 (1.01–1.13)	**89**	**0.0027**	1
HMG20A	rs7178572	G/A	0.64	0.50 (1,343)	0.44 (1,390)	0.45 (447)	**0.0480**	**1.13 (1.00–1.28)**	0.67	1.07 (1.02–1.12)	0	0.4054	9
ZFAND6	rs11634397	G/A	0.56	0.45 (1,000)	0.48 (1,614)	0.46 (601)	0.6605	0.97 (0.86–1.10)	0.64	1.06 (1.04–1.08)	47	0.1706	2
AP3S2	rs2028299	C/A	0.06	0.54 (19)	0.49 (329)	0.46 (2,845)	0.3632	1.12 (0.88–1.42)	0.27	1.05 (1.01–1.09)	0	0.6118	9
PRC1	rs8042680	A/C	0.99	0.47 (3,231)	0.26 (49)	— (0)	**0.0497**	**2.48 (1.00–6.12)**	0.26	1.07 (1.04–1.10)	70	0.0695	2
FTO	rs8050136	A/C	0.14	0.67 (79)	0.48 (806)	0.46 (2,475)	**0.0116**	**1.23 (1.05–1.45)**	0.46	1.14 (1.07–1.21)	0	0.3812	1
SRR	rs391300	C/T	0.83	0.46 (2,326)	0.47 (904)	0.44 (83)	0.8485	0.99 (0.84–1.15)	0.63	1.02 (0.95–1.09)	0	0.6843	1
SLC16A11	rs75493593	T/G	0.41	0.49 (527)	0.47 (1,525)	0.45 (1,071)	0.2168	1.08 (0.96–1.22)	0.01	1.25 (1.18–1.32)*	**78**	**0.0328**	16
TCF2	rs4430796	G/A	0.29	0.47 (295)	0.50 (1,310)	0.44 (1,589)	0.0830	1.12 (0.99–1.28)	0.51	1.10 (1.05–1.15)	0	0.7855	15
HNF4A	rs6017317	G/T	0.83	0.47 (2,229)	0.47 (944)	0.40 (67)	0.6554	1.04 (0.88–1.22)	0.18	1.10 (1.02–1.19)	0	0.5178	1
DUSP9	rs5945326	A/G	0.27	0.46 (473)	0.49 (726)	0.46 (2,014)	0.7378	1.02 (0.91–1.15)	0.77	1.27 (1.18–1.37)	**89**	**0.0024**	2

Alleles are listed with the risk allele given first. The proportion of individuals with T2DM is given among those homozygous for the risk allele (RR), heterozygous individuals (LR), and those homozygous for the low-risk allele (LL). The OR is given per copy of the risk allele as defined in previous studies. Frequencies for Europeans are derived from the HapMap CEU population, and European ORs are derived from the reference. I^2 represents the percentage of variance in the ORs attributable to heterogeneity between Pimas and Europeans, and P_{het} is the *P* value for the null hypothesis that the two ORs are equal. Results with nominal statistical significance ($P < 0.05$) are shown in boldface type. Results in Pimas are adjusted for age, sex, birth year, and heritage. freq., frequency of the risk allele. *The risk allele for the *SLC16A11* variant rs75493593 is rare in Europeans, so the "global" OR is reported (16).

Table 2—Variants with significant ($P < 0.05$) and directionally consistent associations with metabolic traits

Associated with insulin secretion				Mean acute insulin response (μU/mL)					
Gene	SNP	R/L	Freq.	RR	LR	LL	*P*	Eff (95% CI)	*r*
PROX1	rs340874	C/T	0.35	150 (26)	229 (141)	234 (113)	0.0121	0.87 (0.78–0.97)	−0.14
IGF2BP2	rs4402960	T/G	0.17	186 (7)	194 (63)	234 (176)	0.0272	0.85 (0.74–0.98)	−0.17
ZBED3*	rs4457053	G/A	0.36	209 (38)	199 (122)	249 (117)	0.0221	0.88 (0.80–0.98)	−0.16
DGKB-TMEM195	rs2191349	T/G	0.19	175 (12)	190 (101)	248 (173)	0.0002	0.79 (0.70–0.89)	−0.24
GLIS3	rs7041847	A/G	0.66	203 (99)	223 (146)	276 (40)	0.0080	0.87 (0.78–0.96)	−0.16
CDC123	rs12779790	G/A	0.15	162 (7)	200 (56)	231 (204)	0.0382	0.85 (0.74–0.99)	−0.09
HHEX	rs1111875	C/T	0.39	179 (35)	224 (128)	238 (93)	0.0272	0.88 (0.79–0.99)	−0.12
KCNQ1*	rs2237892	C/T	0.51	180 (65)	231 (135)	249 (79)	0.0019	0.85 (0.77–0.94)	−0.20
MNTR1B	rs1387153	T/C	0.11	320 (2)	174 (41)	230 (235)	0.0405	0.82 (0.68–0.99)	−0.15
Associated with insulin sensitivity				Mean M (mg/kg EMBS/min)					
Gene	SNP	R/L	Freq.	RR	LR	LL	*P*	Eff (95% CI)	*r*
IRS1	rs7578326	A/G	0.91	3.48 (330)	3.71 (55)	4.16 (2)	0.0263	0.94 (0.88–0.99)	−0.12
PPARG	rs1801282	C/G	0.92	3.47 (331)	3.80 (58)	3.65 (5)	0.0087	0.93 (0.88–0.98)	−0.12
MNTR1B	rs1387153	T/C	0.11	2.56 (2)	3.40 (67)	3.55 (319)	0.0411	0.94 (0.89–1.00)	−0.11
PRC1*	rs8042680	A/C	0.99	3.51 (383)	4.52 (6)	— (0)	0.0179	0.78 (0.63–0.96)	−0.12
SRR	rs391300	C/T	0.83	3.45 (291)	3.71 (92)	3.82 (10)	0.0028	0.94 (0.90–0.98)	−0.15
Associated with Adiposity				Mean Percentage Body Fat (%)					
Gene	SNP	R/L	Freq.	RR	LR	LL	*P*	Eff (95% CI)	*r*
PRC1*	rs8042680	A/C	0.99	32.2 (383)	24.0 (6)	— (0)	0.0087	1.35 (1.08–1.68)	0.13
ZFAND3*	rs9470794	C/T	0.03	— (0)	36.2 (16)	31.7 (378)	0.0314	1.14 (1.01–1.29)	0.12

Directionally consistent associations are those for which the T2DM risk allele is associated with lower insulin secretion, lower insulin sensitivity, or higher percentage body fat. R/L represents the risk and low-risk alleles for T2DM, respectively, based on previously published studies. RR, LR, and LL are the geometric means for each variable in individuals homozygous for the risk allele and heterozygous and homozygous for the low-risk allele, respectively. The logarithm of each variable was analyzed and the regression coefficient was exponentiated to obtain the effect (Eff) per copy of the T2DM risk allele, expressed as a multiplier. *R* is the correlation between the trait and the risk allele. Results for insulin secretion are adjusted for age, sex, heritage, insulin sensitivity, and percentage body fat. Results for insulin sensitivity are adjusted for age, sex, heritage, and percentage body fat. Results for percentage body fat are adjusted for age, sex, and heritage. Freq., frequency of the risk allele in Pimas. *Risk allele is significantly associated with T2DM in Pimas. (See Table 1.)

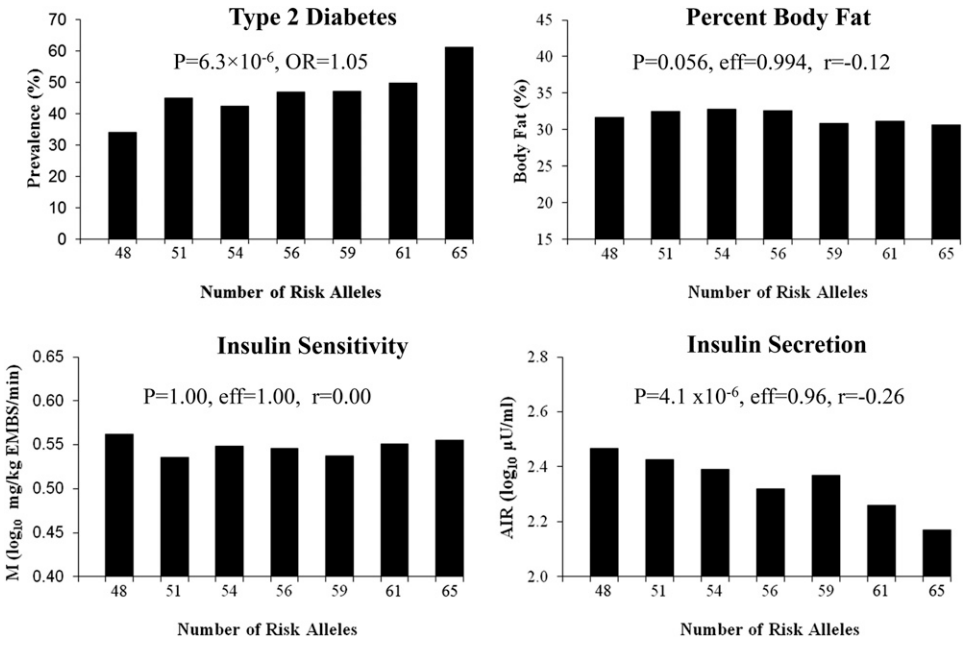

Figure 2—Relationship between the GRS across 55 loci and prevalence of T2DM, percentage body fat, insulin sensitivity, and insulin secretion in Pima Indians. The GRS is calculated as the number of risk alleles and shown in categories, plotted at the midpoint. Results for T2DM involve 3,247 individuals and are adjusted for age, sex, birth year, and heritage. Results for percentage body fat involve 384 individuals and are adjusted for age, sex, and heritage. Results for insulin sensitivity involve 384 individuals and are adjusted for age, sex, heritage, and percentage body fat. Results for insulin secretion involve 274 individuals and are adjusted for age, sex, heritage, percentage body fat, and insulin sensitivity. ORs calculated per copy of a risk allele. AIR, acute insulin response. eff, effect.

Population Risk Attributable to T2DM Loci

Results for the calculation of GAF for Pimas compared with Europeans are shown in Fig. 5*A*. The age-sex adjusted prevalence of T2DM was 48.2% in Pima Indians and 8.2% in non-Hispanic whites from NHANES (OR 10.5). The prevalence in Pimas adjusted to the frequency of risk alleles in CEU was slightly higher at 55.9%, resulting in a GAF of −0.19 (95% CI −0.34, −0.03); the low value of GAF reflects the lower value of the GRS in Pimas. When the 10 SNPs with statistically significant heterogeneity in the ORs were excluded from the calculation, GAF was −0.03 (−0.19, 0.08). Calculations were also conducted comparing non-Hispanic blacks in NHANES (n = 1,610, mean ± SD age 39.5 ± 20.1 years, 812 with diabetes) with non-Hispanic whites using allele frequencies derived from the African ancestry in the southwest U.S. (ASW) HapMap population. These analyses suggest that 66% of the excess prevalence in the black population is potentially attributable

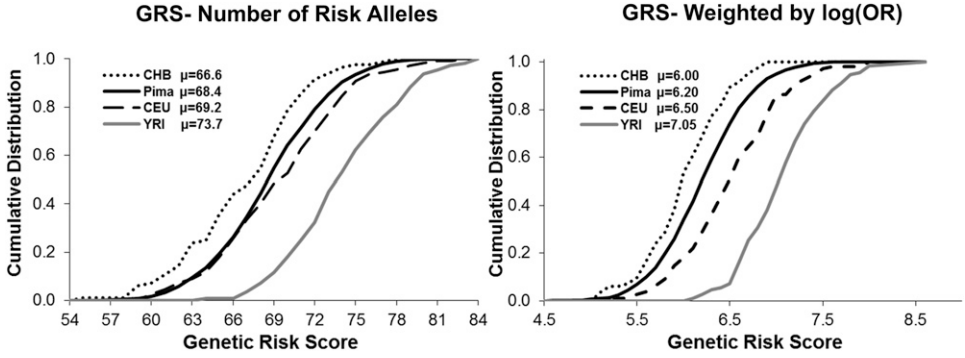

Figure 3—Cumulative distribution of the GRS for T2DM in Pimas and in each of the HapMap populations (CHB, CEU, and YRI). In the left panel, the GRS was calculated as the sum of the number of risk alleles across all 63 loci, while in the right panel it is the sum of the number of risk alleles multiplied by log(OR), as determined in Europeans. Differences in the mean GRS (µ) between populations were compared in a mixed model in which population was a fixed effect and sibship was a random effect. P values for comparison of the GRS between populations are as follows: $P = 0.049$ (1.2×10^{-10}) for Pimas and CEU, $P = 9.6 \times 10^{-5}$ (1.5×10^{-4}) for Pimas and CHB, $P = 9.4 \times 10^{-38}$ (2.5×10^{-72}) for Pimas and YRI, $P = 9.7 \times 10^{-5}$ (1.3×10^{-22}) for CEU and CHB, $P = 7.9 \times 10^{-13}$ (5.4×10^{-15}) for CEU and YRI, and $P = 1.5 \times 10^{-23}$ (5.7×10^{-46}) for CHB and YRI, where the value in parentheses is for the GRS weighted by log(OR). Results involve 3,253 Pimas, 110 individuals for CEU, 84 individuals for CHB, and 111 individuals for YRI. (For HapMap populations, calculation was restricted to founders.)

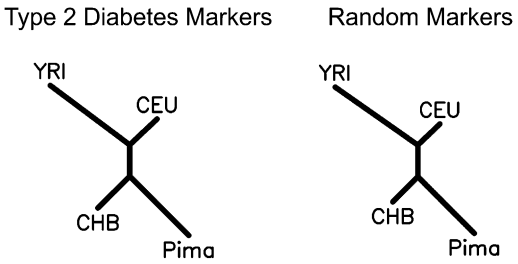

Figure 4—Dendrogram summarizing genetic distances between Pima Indians and HapMap populations at 63 T2DM susceptibility loci and at randomly selected genomic markers. Genetic distance was calculated as F_{ST}, and the dendrogram was generated using PHYLIP. F_{ST} is calculated as the mean value from a bootstrap procedure in which a matching random SNP was selected for each T2DM SNP from a GWAS. (See text for details.) F_{ST} values for T2DM/random markers are as follows: 0.138/0.129 (P = 0.79 for difference) for Pima and CHB, 0.163/0.158 (P = 0.83) for Pima and CEU, 0.232/0.241 (P = 0.80) for Pima and YRI, 0.147/0.146 (P = 0.99) for CEU and YRI, 0.187/0.185 (P = 0.95) for CHB and YRI, and 0.123/0.109 (P = 0.53) for CEU and CHB.

to allele frequency differences at these loci (GAF 0.66 [95% CI 0.32, 1.07]) (Figure 5B).

DISCUSSION

In recent years, many genetic variants reproducibly associated with T2DM have been identified. These have mostly been identified by GWAS in European populations. Many of these variants are also associated with T2DM in non-European populations, but there are instances of heterogeneity (8,10,20). The extent of association in high-risk populations, such as American Indians, is not well characterized. Our previous analyses in Pima Indians, with a much smaller number of SNPs, identified associations with SNPs in *FTO* and *KCNQ1* (27,28); the *KCNQ1* associations are subject to parent-of-origin effects and are particularly strong in Pimas (28). *KLF14* variants also show parent-of-origin effects (28). Statistically significant heterogeneity between Pimas and Europeans at *TCF7L2* was also observed, and a multiallelic score from eight SNPs was modestly associated with T2DM in Pimas and with diminished insulin secretion (20,27). In the present study, we have conducted a more complete survey of T2DM susceptibility variants in Pimas, including a total of 63 SNPs reproducibly associated with T2DM at genome-wide significance. These analyses identify additional SNPs that are nominally significantly associated with T2DM in Pimas in the same direction as in Europeans, including those in *GCKR, ZBED3, CDKAL1, ZFAND3, SPRY2, HMG20A*, and *PRC1*. Many of the T2DM susceptibility SNPs have effects in Pimas that are directionally consistent with those in Europeans, even if they were not individually statistically significant. Indeed, a multiallelic GRS that assesses effects of these variants in aggregate was statistically significant, even when SNPs with nominally significant effects or heterogeneity were excluded. The GRS was also strongly associated with diminished insulin secretion. Thus, the present findings suggest that the majority of T2DM-susceptibility variants do have modest effects on T2DM in this high-risk population but that some do not achieve statistical significance in the current sample size. Analyses in European populations suggest that the majority of T2DM-susceptibility variants influence T2DM risk through an effect on insulin secretion (2,47), and the current analyses suggest that this is also the case in Pimas.

Despite general consistency for most SNPs between the direction of association with T2DM in Pimas and that observed in the original GWAS, there were several SNPs that showed evidence for heterogeneity in effect between Pimas and Europeans. In addition to *TCF7L2*, nominally significant heterogeneity was observed at *IRS1*, *ADAMTS9*, *ARL15*, *ZFAND3*, *PTPRD*, *C2CD4A*, *MPHOSPH9*, *SLC16A11*, and *DUSP9*. With the exception of *ZFAND3*, which has previously been described as associated in East Asians but not Europeans (8), the effect in Pimas was weaker than that in Europeans. Furthermore, the combined test of

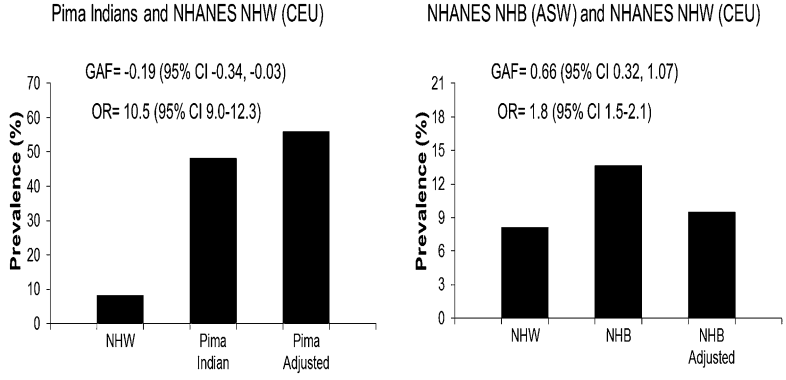

Figure 5—Calculation of the GAF in Pima Indians compared with non-Hispanic whites (NHW) from NHANES (left panel) and NHANES non-Hispanic blacks (NHB) compared with non-Hispanic whites (right panel). The age- and sex-adjusted prevalence of T2DM is shown on the *y*-axis for each group. The "adjusted" value represents the age- and sex-adjusted prevalence for the target population adjusted to the frequency of the risk alleles in the reference population across all 63 loci. The calculation comparing non-Hispanic blacks with non-Hispanic whites uses frequencies from the African ancestry in the southwest U.S. HapMap population, which may be more representative of African Americans than YRI.

heterogeneity across all loci indicated that effects were generally weaker in Pimas than in Europeans (even when SNPs with nominally significant association or heterogeneity were excluded). Thus, while most T2DM-susceptibility variants do have an effect on T2DM risk in Pimas, this effect is generally not as strong as it is in Europeans. It is possible that, despite the large sample sizes, this heterogeneity reflects overestimation of effects in Europeans. Given that functional variants at most of these loci have not been identified, however, some heterogeneity between Europeans and other populations might be expected on account of differing linkage disequilibrium patterns. Indeed, fine-mapping studies have suggested that population heterogeneity at GWAS signals derived from Europeans is at least partly due to differences in linkage disequilibrium patterns (18).

Recent studies have described divergence in allele frequency at T2DM-susceptibility variants between major continental populations that is greater than expected given genetic distances between these populations and a gradient in genetic risk for T2DM with risk alleles at highest frequency in Africans and at lowest frequency in East Asians (48,49). Such divergence in allele frequencies may reflect effects of natural selection in the different evolutionary histories of these populations. Prevalence of T2DM among Pima Indians is among the highest reported in the world, and if such evolutionary factors are responsible for this high prevalence, one might expect to see established T2DM risk alleles at high frequency in Pimas. The present analyses are consistent with previous studies, conducted with fewer SNPs (48), in that we observed the highest genetic risk scores in Africans (YRI) and the lowest in East Asians (CHB). However, genetic risk scores in Pimas were not particularly high and were comparable with, or lower than, those from low-risk populations, such as Europeans. The population differences in GRS observed here could reflect effects of genetic drift or natural selection. One study found that the Africa–East Asia gradient was greater than expected with random markers (49), which suggests natural selection, but a recent study that analyzed several global populations at 65 established T2DM-susceptibility loci suggested that T2DM-susceptibility alleles are generally evolutionarily neutral (50). Further work is needed to determine whether the high genetic risk scores for T2DM in African versus Asian populations is reflective of genetic drift or natural selection. However, in the present general analysis of genetic distances, we did not observe significant excess in the divergence between Pimas and other continental populations across established T2DM-susceptibility variants. This suggests that any overall effects of natural selection at these variants do not appear to have contributed to the high risk of T2DM in Pimas.

Regardless of the mechanisms by which population differences in risk allele frequency have arisen, such differences could explain population differences in prevalence of T2DM. The present analyses of GAF, however, suggest that differences in allele frequency at these established T2DM variants account for little of the increased population risk for T2DM in Pimas compared with European Americans. GWAS within Amerindian-derived populations may identify variants that are more likely to explain these population differences. Our recent GWAS comparing Pimas with young-onset T2DM to older nondiabetic individuals found association with a variant in *DNER* in Pimas but not in Europeans (45); this variant (rs1861612) shows little difference in allele frequency, however. A recent study suggested that the risk allele of rs75493593 in *SLC16A11*, which is more common in American Indians than Europeans, could explain ~20% of the excess risk in Mexican Americans compared with European Americans, ignoring the effects of all other loci (16). In the present study, we found that the risk allele at *SLC16A11* is much more common in Pima Indians than in Europeans; however, its effect is outweighed by other variants at which the risk allele is less common Pimas, such that the overall extent to which established T2DM risk alleles can account for the excess prevalence in Pimas is negligible. In contrast, the present analyses suggest that 66% of the difference in T2DM prevalence between African Americans and European Americans is potentially attributable to allele frequency differences at these loci. Since transferability of European-derived T2DM variants to African Americans is somewhat uncertain given the highly divergent linkage disequilibrium patterns, the validity of the assumption that European-derived ORs represent causal effects may be questionable. Nonetheless, in light of the high proportion of excess prevalence between African Americans and Europeans that is attributable to differences in allele frequency at established T2DM variants, the fact that they account for none of the excess T2DM prevalence in Pimas seems remarkable.

In summary, the present analyses suggest that established T2DM variants are largely transferrable to high-risk populations, such as Pima Indians, albeit with weaker effects than in Europeans. However, differences in allele frequency across these established T2DM alleles account for little, if any, of the high T2DM prevalence in Pimas compared with populations of European ancestry. Thus, the high prevalence of T2DM in Pimas is likely the result of environmental factors or of genetic factors that remain largely unidentified.

Acknowledgments. The authors thank the participants who volunteered for the study and the staff of the Phoenix Epidemiology and Clinical Research Branch who provided assistance.

Funding. This work was supported by the intramural research program of the National Institute of Diabetes and Digestive and Kidney Diseases.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. R.L.H. wrote the manuscript, researched data, and contributed to discussion. R.R., S.K., Y.L.M., E.J.W., J.M.C., R.G.N., and L.J.B. researched data, contributed to discussion, and reviewed and edited the manuscript. R.L.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the Annual Meeting of the American Society of Human Genetics, San Francisco, CA, 6–10 November 2012.

References

1. Zeggini E, Scott LJ, Saxena R, et al.; Wellcome Trust Case Control Consortium. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2008; 40:638–645

2. Voight BF, Scott LJ, Steinthorsdottir V, et al.; MAGIC investigators; GIANT Consortium. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 2010;42:579–589

3. Morris AP, Voight BF, Teslovich TM, et al.; Wellcome Trust Case Control Consortium; Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) Investigators; Genetic Investigation of ANthropometric Traits (GIANT) Consortium; Asian Genetic Epidemiology Network–Type 2 Diabetes (AGEN-T2D) Consortium; South Asian Type 2 Diabetes (SAT2D) Consortium; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet 2012;44:981–990

4. Unoki H, Takahashi A, Kawaguchi T, et al. SNPs in *KCNQ1* are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet 2008;40:1098–1102

5. Yasuda K, Miyake K, Horikawa Y, et al. Variants in *KCNQ1* are associated with susceptibility to type 2 diabetes mellitus. Nat Genet 2008;40:1092–1097

6. Tsai FJ, Yang CF, Chen CC, et al. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. PLoS Genet 2010;6:e1000847

7. Shu XO, Long J, Cai Q, et al. Identification of new genetic risk variants for type 2 diabetes. PLoS Genet 2010;6:e1001127

8. Cho YS, Chen CH, Hu C, et al.; DIAGRAM Consortium; MuTHER Consortium. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet 2012;44:67–72

9. Kooner JS, Saleheen D, Sim X, et al.; DIAGRAM; MuTHER. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nat Genet 2011;43:984–989

10. Saxena R, Saleheen D, Been LF, et al.; DIAGRAM; MuTHER; AGEN. Genome-wide association study identifies a novel locus contributing to type 2 diabetes susceptibility in Sikhs of Punjabi origin from India. Diabetes 2013; 62:1746–1755

11. Saxena R, Elbers CC, Guo Y, et al.; Look AHEAD Research Group; DIAGRAM consortium. Large-scale gene-centric meta-analysis across 39 studies identifies type 2 diabetes loci. Am J Hum Genet 2012;90:410–425

12. Tabassum R, Chauhan G, Dwivedi OP, et al.; DIAGRAM; INDICO. Genome-wide association study for type 2 diabetes in Indians identifies a new susceptibility locus at 2q21. Diabetes 2013;62:977–986

13. Palmer ND, McDonough CW, Hicks PJ, et al.; DIAGRAM Consortium; MAGIC Investigators. A genome-wide association search for type 2 diabetes genes in African Americans. PLoS ONE 2012;7:e29202

14. Dupuis J, Langenberg C, Prokopenko I, et al.; DIAGRAM Consortium; GIANT Consortium; Global BPgen Consortium; Anders Hamsten on behalf of Procardis Consortium; MAGIC investigators. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 2010;42:105–116

15. Gudmundsson J, Sulem P, Steinthorsdottir V, et al. Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. Nat Genet 2007;39:977–983

16. Williams AL, Jacobs SB, Moreno-Macías H, et al.; SIGMA Type 2 Diabetes Consortium. Sequence variants in SLC16A11 are a common risk factor for type 2 diabetes in Mexico. Nature 2014;506:97–101

17. Mahajan A, Go MJ, Zhang W, et al.; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium; Asian Genetic Epidemiology Network Type 2 Diabetes (AGEN-T2D) Consortium; South Asian Type 2 Diabetes (SAT2D) Consortium; Mexican American Type 2 Diabetes (MAT2D) Consortium; Type 2 Diabetes Genetic Exploration by Nex-generation sequencing in muylti-Ethnic Samples (T2D-GENES) Consortium. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet 2014;46:234–244

18. Carlson CS, Matise TC, North KE, et al.; PAGE Consortium. Generalization and dilution of association results from European GWAS in populations of non-European ancestry: the PAGE study. PLoS Biol 2013;11:e1001661

19. Ng MC, Saxena R, Li J, et al. Transferability and fine mapping of type 2 diabetes loci in African Americans: the Candidate Gene Association Resource Plus Study. Diabetes 2013;62:965–976

20. Guo T, Hanson RL, Traurig M, et al. TCF7L2 is not a major susceptibility gene for type 2 diabetes in Pima Indians: analysis of 3,501 individuals. Diabetes 2007;56:3082–3088

21. Knowler WC, Pettitt DJ, Saad MF, Bennett PH. Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. Diabetes Metab Rev 1990;6:1–27

22. American Diabetes Association Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–1197

23. Bunt JC, Krakoff J, Ortega E, Knowler WC, Bogardus C. Acute insulin response is an independent predictor of type 2 diabetes mellitus in individuals with both normal fasting and 2-h plasma glucose concentrations. Diabetes Metab Res Rev 2007;23:304–310

24. Tian C, Hinds DA, Shigeta R, et al. A genomewide single-nucleotide-polymorphism panel for Mexican American admixture mapping. Am J Hum Genet 2007;80:1014–1023

25. Hanis CL, Chakraborty R, Ferrell RE, Schull WJ. Individual admixture estimates: disease associations and individual risk of diabetes and gallbladder disease among Mexican-Americans in Starr County, Texas. Am J Phys Anthropol 1986;70:433–441

26. Muller YL, Bogardus C, Beamer BA, Shuldiner AR, Baier LJ. A functional variant in the peroxisome proliferator-activated receptor gamma2 promoter is associated with predictors of obesity and type 2 diabetes in Pima Indians. Diabetes 2003;52:1864–1871

27. Rong R, Hanson RL, Ortiz D, et al. Association analysis of variation in/near FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761, and CDKN2B with type 2 diabetes and related quantitative traits in Pima Indians. Diabetes 2009;58: 478–488

28. Hanson RL, Guo T, Muller YL, et al. Strong parent-of-origin effects in the association of *KCNQ1* variants with type 2 diabetes in American Indians. Diabetes 2013;62:2984–2991

29. Nair AK, Muller YL, McLean NA, et al. Variants associated with type 2 diabetes identified by the transethnic meta-analysis study: assessment in American Indians and evidence for a new signal in LPP. Diabetologia 2014; 57:2334–2338

30. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–560

31. Gouda HN, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JP. The association between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: a HuGE review and meta-analysis. Am J Epidemiol 2010;171:645–655

32. Zhao Y, Ma YS, Fang Y, et al. IGF2BP2 genetic variation and type 2 diabetes: a global meta-analysis. DNA Cell Biol 2012;31:713–720

33. Franks PW, Rolandsson O, Debenham SL, et al. Replication of the association between variants in WFS1 and risk of type 2 diabetes in European populations. Diabetologia 2008;51:458–463

34. Dehwah MA, Wang M, Huang QY. CDKAL1 and type 2 diabetes: a global meta-analysis. Genet Mol Res 2010;9:1109–1120

35. Cauchi S, Del Guerra S, Choquet H, et al. Meta-analysis and functional effects of the SLC30A8 rs13266634 polymorphism on isolated human pancreatic islets. Mol Genet Metab 2010;100:77–82

36. Cugino D, Gianfagna F, Santimone I, et al. Type 2 diabetes and polymorphisms on chromosome 9p21: a meta-analysis. Nutr Metab Cardiovasc Dis 2012;22:619–625
37. Cai Y, Yi J, Ma Y, Fu D. Meta-analysis of the effect of HHEX gene polymorphism on the risk of type 2 diabetes. Mutagenesis 2011;26:309–314
38. Tong Y, Lin Y, Zhang Y, et al. Association between TCF7L2 gene polymorphisms and susceptibility to type 2 diabetes mellitus: a large Human Genome Epidemiology (HuGE) review and meta-analysis. BMC Med Genet 2009;10:15
39. Liu J, Wang F, Wu Y, et al. Meta-analysis of the effect of KCNQ1 gene polymorphism on the risk of type 2 diabetes. Mol Biol Rep 2013;40:3557–3567
40. Gong B, Yu J, Li H, Li W, Tong X. The effect of KCNJ11 polymorphism on the risk of type 2 diabetes: a global meta-analysis based on 49 case-control studies. DNA Cell Biol 2012;31:801–810
41. Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 1984;36:460–465
42. Stouffer SA, Suchman EA, DeVinney LC, Star SA, Williams RM. How the volumes were produced. In *The American Soldier, Volume 1: Adjustment During Army Life.* Princeton, NJ, Princeton University Press, 1949, p 45
43. Weir BS, Hill WG. Estimating F-statistics. Annu Rev Genet 2002;36:721–750
44. Yang Y, Remmers EF, Ogunwole CB, Kastner DL, Gregersen PK, Li W. Effective sample size: Quick estimation of the effect of related samples in genetic case-control association analyses. Comput Biol Chem 2011;35:40–49
45. Hanson RL, Muller YL, Kobes S, et al. A genome-wide association study in American Indians implicates DNER as a susceptibility locus for type 2 diabetes. Diabetes 2014;63:369–376
46. Kooperberg C, Petitti DB. Using logistic regression to estimate the adjusted attributable risk of low birthweight in an unmatched case-control study. Epidemiology 1991;2:363–366
47. Jonsson A, Ladenvall C, Ahluwalia TS, et al. Effects of common genetic variants associated with type 2 diabetes and glycemic traits on α- and β-cell function and insulin action in humans. Diabetes 2013;62:2978–2983
48. Chen R, Corona E, Sikora M, et al. Type 2 diabetes risk alleles demonstrate extreme directional differentiation among human populations, compared to other diseases. PLoS Genet 2012;8:e1002621
49. Corona E, Chen R, Sikora M, et al. Analysis of the genetic basis of disease in the context of worldwide human relationships and migration. PLoS Genet 2013;9:e1003447
50. Ayub Q, Moutsianas L, Chen Y, et al. Revisiting the thrifty gene hypothesis via 65 loci associated with susceptibility to type 2 diabetes. Am J Hum Genet 2014;94:176–185