Strong Parent-of-Origin Effects in the Association of *KCNQ1* Variants With Type 2 Diabetes in American Indians

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Parent-of-origin effects were observed in an Icelandic population for several genetic variants associated with type 2 diabetes, including those in *KLF14* (rs4731702), *MOB2* (rs2334499), and *KCNQ1* (rs2237892, rs231362). We analyzed parent-of-origin effects for these variants, along with two others in *KCNQ1* identified in previous genome-wide association studies (rs2237895, rs2299620), in 7,351 Pima Indians from 4,549 nuclear families; 34% of participants had diabetes. In a subset of 287 normoglycemic individuals, acute insulin secretion was measured by an intravenous glucose tolerance test. Statistically significant ($P < 0.05$) parent-of-origin effects were seen for association with type 2 diabetes for all variants. The strongest effect was seen at rs2299620 in *KCNQ1*; the C allele was associated with increased diabetes when maternally derived (odds ratio [OR], 1.92; $P = 4.1 \times 10^{-12}$), but not when paternally derived (OR, 0.93; $P = 0.47$; $P = 9.9 \times 10^{-6}$ for difference in maternal and paternal effects). A maternally derived C allele also was associated with a 28% decrease in insulin secretion ($P = 0.002$). This study confirms parent-of-origin effects in the association with type 2 diabetes for variants in *KLF14*, *MOB2*, and *KCNQ1*. In Pima Indians, the effect of maternally derived *KCNQ1* variants appears to be mediated through decreased insulin secretion and is particularly strong, accounting for 4% of the variance in liability to diabetes. ***Diabetes*** **62:2984–2991, 2013**

Several single nucleotide polymorphisms (SNPs) reproducibly associated with type 2 diabetes recently have been identified (1–4). Many of these are in regions of the genome that are imprinted, and studies of an Icelandic population suggest that there are parent-of-origin effects at four of these variants (5); in other words, the extent of association with the risk allele depends on whether it is inherited from the mother or from the father. The SNPs for which parent-of-origin effects have been observed include one in *KLF14* (rs4731702), one near *MOB2* (rs2334499), and two independent SNPs in *KCNQ1* (rs231362 and rs2237892) (5). The presence of parent-of-origin effects at these SNPs is consistent with imprinting and may have important implications for the mechanisms by which variants in or near these genes confer susceptibility to type 2 diabetes. However, for some of the SNPs, the current statistical evidence for parent-of-origin effects is modest. Furthermore, to our knowledge, these effects have not been replicated in other ethnic groups, nor have parent-of-origin effects been analyzed for metabolic traits that underlie the risk of type 2 diabetes, perhaps because few large studies have family data. In the current study, we have analyzed parent-of-origin effects at these SNPs in Pima Indians, an American Indian population in which the prevalence of type 2 diabetes is extraordinarily high (6) and in which family and detailed metabolic data were obtained.

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RESEARCH DESIGN AND METHODS

Participants. The participants were derived from a longitudinal, population-based, epidemiologic study conducted in the Gila River Indian Community in central Arizona, where most of the residents are Pima Indians (6). In this study, all community members older than 5 years of age were invited to attend an examination every 2 years. Information on family relationships was collected. The examinations included a 75-g oral glucose tolerance test. Diabetes was diagnosed according to the 1997 American Diabetes Association criteria (7) (a fasting plasma glucose ≥7.0 mmol/L or a 2-h postload plasma glucose ≥11.1 mmol/L or a diagnosis made during the course of routine clinical care). The current study was conducted in 7,351 individuals whose self-reported heritage was at least half American Indian, who had DNA available, and who had data regarding presence of type 2 diabetes. This included 3,604 individuals (2,061 women and 1,543 men) whose heritage was full Pima or Tohono O'odham (a closely related tribe); the mean (±SD) age of these individuals was 40.0 (±16.7) years and 46% had diabetes. The remaining 3,747 individuals (2,052 women and 1,695 men; mean age, 28.4 ± 14.1 years; 22% with diabetes) were largely of "mixed" heritage; on average, the self-reported heritage of this group was 56% Pima Indian and 83% American Indian (which includes other tribes). The full-heritage Pima Indians and the mixed-heritage individuals were initially analyzed separately, and similar association results were obtained. Thus, results are presented for the combined analysis.

Exposure to a diabetic intrauterine environment is a strong risk factor for development of diabetes in Pima Indians independent of genetic background (8), and such intrauterine effects may confound the analysis of parent-of-origin effects (9). To account for this, individuals were classified into three categories based on their likely exposure to a diabetic intrauterine environment. Individuals whose mothers had diabetes diagnosed before the child's birth were considered to have "definite" exposure; those whose mothers had a nondiabetic examination during the longitudinal study ≥1 year after the child's birth were considered to have "unlikely" exposure to a diabetic intrauterine environment; and the exposure of all others was considered "indeterminate." Covariates representing these categories were included in the analyses of association with diabetes, and separate analyses were conducted for each of these groups.

Metabolic traits. A subset of the population participated in detailed metabolic studies to measure the following traits related to development of diabetes: insulin sensitivity; insulin secretion; and body composition. Percentage body fat was calculated from measurements made by hydrodensitometry or by dual X-ray absorptiometry with use of a conversion equation to make measurements comparable, as previously described (10). These measurements were made in 400 full-heritage Pima Indians (171 women and 229 men; mean age, 26.7 ± 6.1 years). In these same individuals, insulin sensitivity was measured by the hyperinsulinemic-euglycemic clamp technique. As described previously (11), insulin was infused at a rate to approximate physiologic levels (~130 μmol/L) and glucose was infused at a varying rate titrated to maintain normoglycemia. The rate of glucose uptake, estimated using tracer amounts of [3-^{3}H]glucose

and normalized to effective metabolic body size (defined as fat-free mass plus 17.7 kg) (12), was taken as a measure of insulin sensitivity (mg glucose/kg estimated metabolic body size · min). Insulin secretion was measured by a 25-g intravenous glucose tolerance test as the acute insulin response (μU/mL) 3–5 min after the glucose bolus (11) in 287 individuals (104 women and 183 men; mean age, 26.7 ± 6.2 years) with normal glucose tolerance.

Genotypes. DNA was extracted from nuclear pellets derived from buffy coat using a high-salt precipitation method. Genotyping was conducted using SNPplex (Applied Biosystems, Carlsbad, CA), BeadXpress (Illumina, San Diego, CA), or Assays on Demand (Applied Biosystems) methods according to the manufacturer's instructions. Each of the four SNPs reported as showing a parent-of-origin effect in the Icelandic study was genotyped (rs4731702, rs2334499, rs231362, rs2237892). Two additional *KCNQ1* SNPs, rs2237895 and rs2237897, had associations of genome-wide significance with type 2 diabetes in a Japanese population, and had modest to moderate concordance with rs2237892 ($r^2 = 0.30$ in Japanese) (3). Thus, rs2237895 and rs2299620 ($r^2 = 0.97$ with rs2237897 in Pima Indians) also were genotyped in the current study. Analyses of "blind" duplicates and of Hardy-Weinberg equilibrium were performed to ensure genotyping quality; the concordance rate for duplicate genotypes for all SNPs was >97.3% and all had $P > 0.001$ for Hardy-Weinberg equilibrium. In addition, 45 SNPs with large differences in allele frequencies between American Indians and European populations (13) were genotyped to obtain estimates of individual European admixture for use as a covariate in association analyses. Individual admixture estimates were derived from these markers by the method of Hanis et al. (14). Relationships among nuclear family members were confirmed with the PREST program (15) by analysis of up to 1,178 SNPs typed in ongoing genetic studies. The 7,351 individuals constituted 4,549 sibships.

Statistical analysis. The association between genotype and type 2 diabetes was analyzed with control for covariates by a logistic regression model that was fit by the generalized estimating equation procedure to account for dependence among siblings. To assess general association in the absence of parent-of-origin effects, the odds ratio (OR) associated with each copy of the risk allele was calculated from a model in which the number of risk alleles was coded as a numeric variable (0, 1, or 2). The likely parental origin of alleles was assigned by analysis of genotypes observed in an individual, their parents, and their siblings (19% of individuals had both parents available for genotyping, whereas 42% had one parent available and 39% had neither parent available). Two variables, G_M and G_F, representing the presence of a risk allele inherited from the mother (M) or the father (F), respectively, were included in the logistic regression model. For homozygous individuals, these variables can be assigned unambiguously, i.e., $G_M = 1$ and $G_F = 1$ for those homozygous for the risk allele and $G_M = 0$ and $G_F = 0$ for those homozygous for the low-risk allele. For heterozygous individuals with at least one homozygous parent, the variables also can be assigned unambiguously, e.g., $G_M = 1$ and $G_F = 0$ for a heterozygous individual whose mother is homozygous for the risk allele. For heterozygous individuals for whom the assignment was uncertain, G_M and G_F were assigned their expected values. This expected value was calculated from the population allele frequencies and the genotypes of all family members using the MLINK program (16). Supplementary Table 1 shows the assignment of G_M and G_F for various combinations of genotypes for parent and child.

The regression coefficients for the effects of G_M and G_F (β_M and β_F) were used to calculate the ORs associated with the presence of a maternally inherited risk allele (OR_M) and a paternally inherited risk allele (OR_F). A test of parent-of-origin effects was conducted by testing the null hypothesis of equality of these ORs. This was performed by comparison of the difference of $\beta_M - \beta_F$ with its SE, which was derived from the SEs of β_M, β_F, and their covariance as follows

$$\mathrm{SE}(\beta_M - \beta_F) = [\mathrm{SE}(\beta_M)^2 + \mathrm{SE}(\beta_F)^2 - 2\mathrm{cov}(\beta_M, \beta_F)]^{1/2}$$

P_{dif} is the P value associated with this test.

Simulations conducted with the present set of families suggest that this method has levels of type I error that are close to nominal values under the null hypothesis $OR_M = OR_F$ and produces estimates that generally closely approximate the true parent-specific ORs (Supplementary Figs. 1 and 2). In the Icelandic study, the OR for the risk allele inherited from the presumptively expressed parent is ~1.3 (5). The simulations suggest that power to detect an OR of this magnitude at $P < 0.05$ in the present families was 84% for a risk allele with frequency of 0.1 and 99% for a risk allele with frequency of 0.5; powers to detect a parent-of-origin effect at $P_{dif} < 0.05$ were 50 and 70%, respectively.

Analyses of association with continuous variables were conducted in a manner analogous to those for diabetes, with use of a linear mixed model to account for familial effects. The logarithm of each of the traits was analyzed as the dependent variable in these analyses, and the regression coefficient (β) was exponentiated to obtain the average effect on trait values associated with the risk allele, expressed as a multiplier (e.g., $\exp[\beta] = 1.10$ represents an increase of 10% in the trait value for each copy of the risk allele, whereas $\exp[\beta] = 0.90$ represents a decrease of 10%). To examine the potential differential association of diabetes with alleles at multiple SNPs in linkage disequilibrium, the methods were extended to assess the parental origin of haplotypes inferred from the genotypes at two or three SNPs. This allows for analysis of parent-specific association for haplotypes composed of the risk allele at one variant and the low-risk allele at one or two of the others.

Results for association of specific parentally derived alleles with type 2 diabetes were combined across the Pima Indian and Icelandic studies by the inverse variance method (17). The published ORs and P values from the Icelandic study were used in these analyses (5), and these P values were used to infer test-based SEs. Cochran's Q statistic was used to assess heterogeneity between the ORs for the two populations. P values for the parent-of-origin effect were combined across populations by an unweighted Z method (18). The proportion of the variance in liability to diabetes accounted for by an association was estimated by iterative application of formulae that relate this value to the OR, allele frequency, and disease prevalence (19), with modification for parent-of-origin effects.

RESULTS

Combined analysis of Pima Indian and Icelandic populations. Table 1 shows the results of parent-specific association analyses combined across the Pima Indian and Icelandic populations for the four SNPs typed in common. There was a statistically significant parent-of-origin effect ($P_{dif} < 0.05$) for each of the SNPs, and the directions of the parent-specific associations were consistent with those expected from the Icelandic study. In general, the evidence for parent-of-origin effects was strongest when both populations were combined. For the *KCNQ1* SNP rs2237892, the formal evidence for a parent-of-origin effect was marginal in the Icelandic study, in which the high frequency of the risk allele (0.93) resulted in limited power to detect these effects. In the Pima Indians, the risk allele has a frequency near 0.50, and this results in high power to discriminate parental origin; when the Pima and Icelandic populations are combined, the P value for the parent-of-origin effect at rs2237892 is stronger than in the Icelandic population alone ($P_{dif} = 6.4 \times 10^{-4}$). There was little evidence for heterogeneity between the populations, except at the *KCNQ1* SNP rs231362, where there was suggestion of a protective effect of the paternally derived C allele in Pima Indians, but not in the Icelandic population.

Association with type 2 diabetes in Pima Indians. Results for the associations of alleles at each of the six SNPs with type 2 diabetes according to parental origin in Pima Indians are shown in Fig. 1. The additional two *KCNQ1* SNPs derived from the Japanese studies (rs2237895 and rs2299620) also showed significant parent-of-origin effects ($P_{dif} < 0.05$) and strong associations with type 2 diabetes. The strongest associations were seen with the *KCNQ1* SNPs rs2237892, rs2237895, and rs2299620.

Table 2 shows the association in individuals classified according to their likelihood of exposure to a diabetic intrauterine environment. In general, the results were consistent across categories of exposure to diabetes in utero. Although there is some variability for the small group of individuals with definite exposure, there were no statistically significant interactions between exposure categories and parent-specific alleles. Furthermore, the parent-of-origin effects for all SNPs remained statistically significant ($P_{dif} < 0.05$) among those with unlikely exposure to diabetes in utero, with the exception of the *KCNQ1* SNP rs231362, for which $P_{dif} = 0.07$. These analyses indicate that confounding of the parent-of-origin effects by exposure to diabetes in utero is unlikely.

TABLE 1
Meta-analysis of parent-of-origin effects in Pima Indians and the Icelandic population in the association with type 2 diabetes

Gene	SNP	Allele	Population	Frequency	OR_M (95% CI)	P	P_{het}	OR_F (95% CI)	P	P_{het}	P_{dif}
KLF14	rs4731702	C/T	Icelandic	0.44	1.17 (1.07–1.28)	**1.0×10^{-3}**	—	0.99 (0.92–1.07)	0.79	—	**0.022**
—	—	—	Pima Indian	0.42	1.36 (1.13–1.63)	**8.9×10^{-4}**	—	0.94 (0.79–1.12)	0.50	—	**0.019**
—	—	—	Combined		1.21 (1.10–1.32)	**6.3×10^{-5}**	0.15	0.98 (0.92–1.05)	0.61	0.28	**3.8×10^{-3}**
MOB2	rs2334499	T/C	Icelandic	0.41	0.86 (0.78–0.95)	**2.0×10^{-3}**	—	1.35 (1.23–1.48)	**4.7×10^{-10}**	—	**4.1×10^{-11}**
—	—	—	Pima Indian	0.58	0.76 (0.63–0.92)	**4.1×10^{-3}**	—	1.16 (0.96–1.40)	0.12	—	**0.011**
—	—	—	Combined		0.84 (0.76–0.92)	**2.4×10^{-4}**	0.26	1.31 (1.20–1.43)	**3.7×10^{-10}**	0.16	**5.4×10^{-10}**
KCNQ1	rs231362	C/T	Icelandic	0.55	1.23 (1.11–1.36)	**6.2×10^{-5}**	—	0.98 (0.87–1.10)	0.73	—	**3.2×10^{-3}**
—	—	—	Pima Indian	0.90	1.42 (1.07–1.89)	**0.017**	—	0.69 (0.53–0.89)	**3.9×10^{-3}**	—	**1.2×10^{-3}**
—	—	—	Combined		1.25 (1.13–1.38)	**1.3×10^{-5}**	0.36	0.92 (0.83–1.02)	0.13	**0.01**	**4.6×10^{-5}**
KCNQ1	rs2237892	C/T	Icelandic	0.93	1.30 (1.07–1.58)	**8.4×10^{-3}**	—	1.03 (0.88–1.20)	0.71	—	0.054
—	—	—	Pima Indian	0.52	1.69 (1.41–2.03)	**1.3×10^{-8}**	—	0.97 (0.81–1.17)	0.78	—	**6.4×10^{-4}**
—	—	—	Combined		1.50 (1.24–1.81)	**3.7×10^{-5}**	0.05	1.01 (0.89–1.13)	0.92	0.65	**6.4×10^{-4}**

In the allele column, the risk allele as determined in the Icelandic study (5) is given first. Frequency is frequency of the risk allele. OR_M is the OR associated with a maternally derived risk allele and OR_F is the OR associated with a paternally derived risk allele. P_{het} is the P value for the null hypothesis of no heterogeneity in the OR between Pima Indian and Icelandic populations. P_{dif} is the P value for the null hypothesis of no parent-of-origin effect ($OR_M = OR_F$). $P < 0.05$ shown in bold.

Association with metabolic traits. To determine potential physiologic mechanisms associated with diabetes risk for these SNPs, their association with quantitative metabolic traits was analyzed (Table 3). There was an association between the diabetes risk allele (C) of the *KLF14* SNP rs4731702 and lower insulin sensitivity, but the parent-of-origin effect was not statistically significant. The three *KCNQ1* SNPs, rs2237892, rs2237895, and rs2299620, were associated with insulin secretion, such that the diabetes risk allele was associated with lower insulin secretion. These associations were statistically significant when the risk allele was of maternal origin, but not when it was of paternal origin. At rs2299620, for example, a maternally inherited C allele was associated with an acute insulin secretion that was 28% lower than for a maternally inherited T allele ($P = 0.002$)

Haplotype analysis of *KCNQ1* variants. The *KCNQ1* SNPs, rs2237892, rs2237895, and rs2299620, are in stronger linkage disequilibrium in Pima Indians than in other populations. The r^2 between rs2237892 and rs2237895 is 0.64 ($D' = 0.86$), whereas between rs2237892 and rs2299620 it is 0.84 ($D' = 0.98$) and between rs2237895 and rs229620 it is 0.73 ($D' = 0.99$). To determine potential independent associations with diabetes, parent-specific haplotypic associations with diabetes were analyzed for pairs of these SNPs (Fig. 2). SNPs rs2237892 and rs2237895 contributed independently to the association with diabetes because maternally derived haplotypes with the risk allele at one marker, but not the other, had increased odds of diabetes compared with those with the low-risk allele at both markers. However, when haplotypes of rs2237892 or rs2237895 were considered in conjunction with rs2299620, maternally derived haplotypes with the risk allele (C) at rs2299620 had a significantly greater risk of diabetes than those with both low-risk alleles, although there were few individuals with haplotypes that carried the low-risk allele at rs2299620 and the risk allele at one of the other SNPs. Furthermore, in the analysis of haplotypes composed of all three SNPs, all haplotypes containing the C allele at rs2299620 are associated with increased risk of diabetes, regardless of alleles at the other loci, which exist in all possibilities (Fig. 2). Therefore, rs2299620 is the primary association signal in Pima Indians.

DISCUSSION

In recent years a number of genetic variants reproducibly associated with type 2 diabetes have been identified (1–4), and four of these variants across three genes in genomic regions known to be imprinted have been identified as having parent-of-origin effects (5). However, the initial studies demonstrating parent-of-origin effects, which were performed in Iceland, have not been replicated in other populations. Robust demonstration of parent-of-origin effects requires family data for large numbers of individuals with and without diabetes, and few studies have such data. In the present analysis, we replicated the parent-of-origin effects for all four of the variants identified in the Icelandic study in 7,351 Pima Indians, a high-risk population for which family data are available. Our findings suggest that functional variants underlying the observed effects at these loci are subject to imprinting and are important determinants of type 2 diabetes in diverse populations, including non-European populations at high risk for diabetes.

When a child's mother has diabetes during the pregnancy, this exposure to a diabetic intrauterine environment

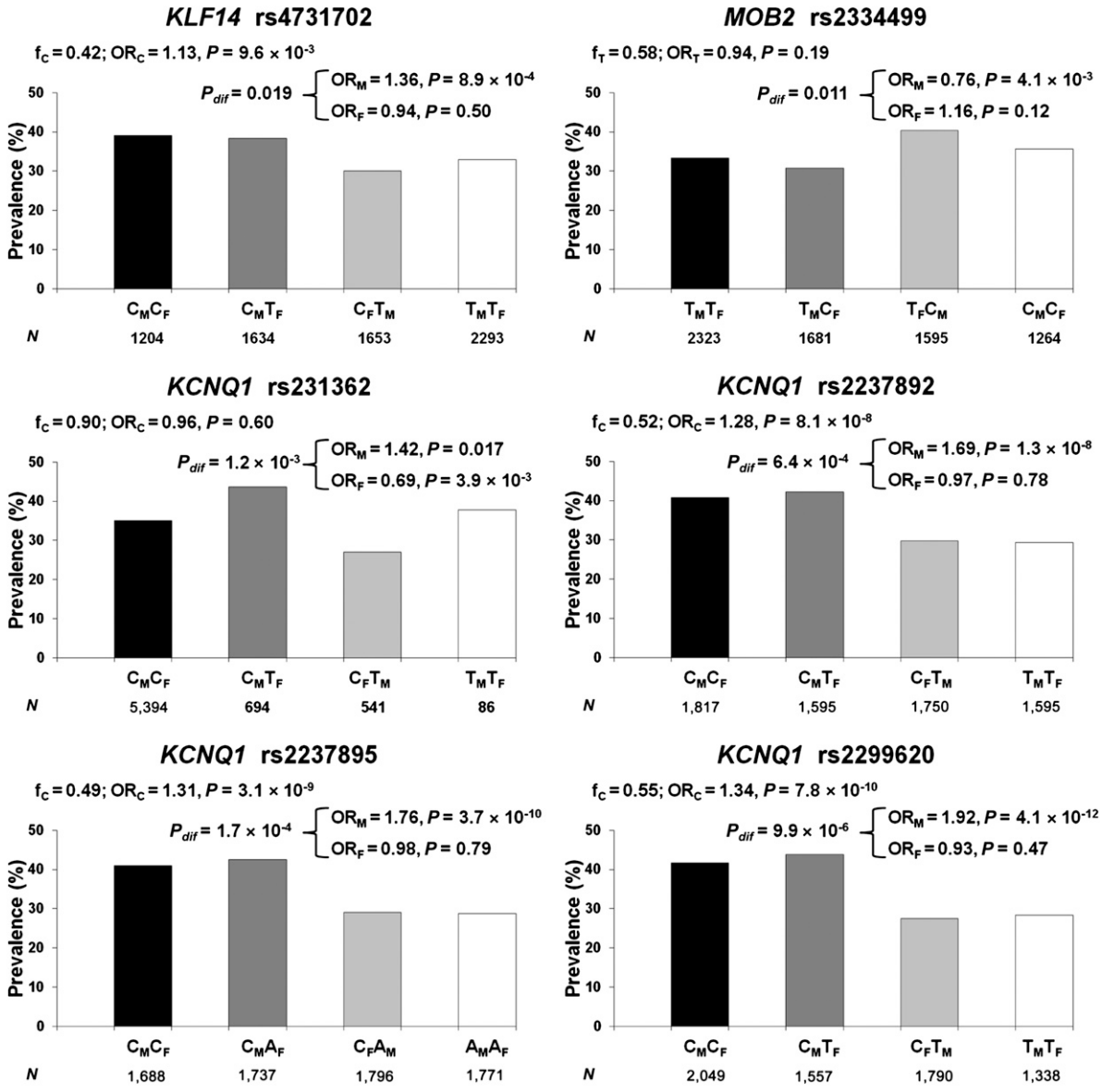

FIG. 1. Prevalence of diabetes according to genotype and parental origin of alleles in American Indians. The SNPs in the *top four panels* were derived from the Icelandic study (5) (see Table 1), whereas rs2237895 and rs2299620 were derived from Japanese studies (3,4), with the latter being a tag for rs2237897. Alleles are designated by base with subscript M or F indicating origin from mother (M) or father (F). OR_i indicates the OR for diabetes per copy of the risk allele i (i = C, T) as determined in the initial study identifying these associations; f_i is the frequency of this risk allele. OR_M is the OR for carriers of a maternally derived risk allele (compared with carriers of a maternally derived low-risk allele), whereas OR_F is the corresponding OR for a paternally derived risk allele. P_{dif} is the P value for the null hypothesis $OR_M = OR_F$. Significant values are indicative of a parent-of-origin effect. All results are adjusted for age, sex, birth year, American Indian heritage, and exposure to diabetes in utero.

increases the child's subsequent risk for type 2 diabetes; such exposure to a diabetic intrauterine environment is an important risk factor for diabetes in Pima Indians (8). In some situations, such intrauterine effects can confound the assessment of parent-of-origin effects (9). However, this does not appear to be the case in the current analyses because the parent-of-origin effects were seen even among those who were not likely exposed to a diabetic intrauterine environment (in that the mother had an examination after the child's birth that did not indicate diabetes).

The consistency of the findings between Pima Indian and Icelandic populations (5) and the consistency with established patterns of imprinting in humans is further evidence that these associations are mediated by imprinted genes. According the Geneimprint website (www.geneimprint.com), *KLF14* and *KCNQ1* are maternally expressed in humans, which is consistent with our observation that the maternally derived risk alleles at variants in these genes are associated with a statistically significant increased risk of diabetes. The SNP rs2334499 is near *MOB2* and the *KRTAP5* cluster, neither of which is known to be imprinted but both are located among the large cluster of imprinted genes on 11p15 (www.geneimprint.com). In this region, the parental alleles appear to act with opposite effect. In the Icelandic study, a strong increased risk of diabetes was noted with inheritance of the paternal risk allele T, whereas the same allele was protective when maternally derived (i.e., C is the risk allele when maternally derived). A similar pattern with respect to maternally derived and paternally derived risk alleles was observed in the current study of Pima Indians. It is possible that this pattern reflects more than one functional variant with

TABLE 2
Association of allelic variants with type 2 diabetes in American Indians according to parental origin and categories of exposure to diabetes in utero

Gene	SNP	Allele	Frequency	OR (95% CI)	P	OR_M (95% CI)	P	OR_F (95% CI)	P	P_{dif}
Unlikely exposure to diabetes in utero (N = 3,438)										
KLF14	rs4731702	C/T	0.41	1.07 (0.92–1.25)	0.38	1.43 (1.10–1.85)	**7.1×10^{-3}**	0.80 (0.62–1.04)	0.094	**6.5×10^{-3}**
MOB2	rs2334499	T/C	0.58	0.94 (0.81–1.09)	0.43	0.70 (0.54–0.91)	**8.0×10^{-3}**	1.28 (0.98–1.68)	0.074	**7.4×10^{-3}**
KCNQ1	rs231362	C/T	0.90	1.09 (0.83–1.43)	0.53	1.46 (0.97–2.20)	0.070	0.86 (0.59–1.26)	0.45	0.070
KCNQ1	rs2237892	C/T	0.51	1.24 (1.07–1.44)	**4.8×10^{-3}**	1.72 (1.33–2.23)	**3.9×10^{-5}**	0.89 (0.68–1.16)	0.38	**2.6×10^{-3}**
KCNQ1	rs2237895	C/A	0.49	1.32 (1.14–1.52)	**2.1×10^{-4}**	1.82 (1.41–2.33)	**2.9×10^{-6}**	0.95 (0.73–1.22)	0.68	**2.0×10^{-3}**
KCNQ1	rs2299620	C/T	0.55	1.35 (1.16–1.57)	**9.4×10^{-5}**	1.96 (1.50–2.55)	**8.1×10^{-7}**	0.93 (0.71–1.21)	0.57	**9.3×10^{-4}**
Indeterminate exposure to diabetes in utero (N = 3,693)										
KLF14	rs4731702	C/T	0.43	1.17 (1.04–1.32)	**0.011**	1.37 (0.99–1.91)	0.059	1.00 (0.72–1.39)	1.00	0.31
MOB2	rs2334499	T/C	0.57	0.96 (0.85–1.08)	0.52	0.92 (0.67–1.27)	0.61	1.00 (0.73–1.39)	0.98	0.78
KCNQ1	rs231362	C/T	0.89	0.92 (0.76–1.13)	0.43	1.59 (0.96–2.63)	0.071	0.56 (0.36–0.89)	**0.013**	**0.019**
KCNQ1	rs2237892	C/T	0.52	1.35 (1.20–1.53)	**1.3×10^{-6}**	1.63 (1.17–2.28)	**3.8×10^{-3}**	1.12 (0.80–1.56)	0.52	0.23
KCNQ1	rs2237895	C/A	0.50	1.33 (1.18–1.50)	**2.2×10^{-6}**	1.72 (1.27–2.34)	**4.7×10^{-4}**	1.03 (0.76–1.40)	0.85	0.075
KCNQ1	rs2299620	C/T	0.56	1.36 (1.20–1.54)	**1.4×10^{-6}**	2.15 (1.57–2.96)	**2.2×10^{-6}**	0.86 (0.63–1.18)	0.35	**2.0×10^{-3}**
Definite exposure to diabetes in utero (N = 220)										
KLF14	rs4731702	C/T	0.43	1.18 (0.77–1.82)	0.45	0.85 (0.41–1.78)	0.67	1.69 (0.78–3.67)	0.18	0.28
MOB2	rs2334499	T/C	0.54	0.65 (0.41–1.03)	0.067	0.70 (0.34–1.43)	0.32	0.60 (0.30–1.23)	0.17	0.80
KCNQ1	rs231362	C/T	0.94	0.63 (0.23–1.70)	0.36	2.42 (0.45–13.1)	0.30	0.26 (0.08–0.85)	**0.025**	**0.030**
KCNQ1	rs2237892	C/T	0.54	1.13 (0.71–1.78)	0.61	1.57 (0.78–3.18)	0.21	0.79 (0.36–1.73)	0.56	0.25
KCNQ1	rs2237895	C/A	0.52	1.33 (0.89–2.01)	0.17	1.77 (0.86–3.62)	0.12	1.00 (0.46–2.18)	0.99	0.37
KCNQ1	rs2299620	C/T	0.57	1.25 (0.80–1.96)	0.33	1.67 (0.76–3.68)	0.20	0.94 (0.41–2.13)	0.87	0.39
All participants (N = 7,351)										
KLF14	rs4731702	C/T	0.42	1.13 (1.03–1.24)	**9.6×10^{-3}**	1.36 (1.13–1.63)	**8.9×10^{-4}**	0.94 (0.79–1.12)	0.50	**0.019**
MOB2	rs2334499	T/C	0.58	0.94 (0.86–1.03)	0.19	0.76 (0.63–0.92)	**4.1×10^{-3}**	1.16 (0.96–1.40)	0.12	**0.011**
KCNQ1	rs231362	C/T	0.90	0.96 (0.82–1.12)	0.60	1.42 (1.07–1.89)	**0.017**	0.69 (0.53–0.89)	**0.0039**	**1.2×10^{-3}**
KCNQ1	rs2237892	C/T	0.52	1.28 (1.17–1.41)	**8.1×10^{-8}**	1.69 (1.41–2.03)	**1.3×10^{-8}**	0.97 (0.81–1.17)	0.78	**6.4×10^{-4}**
KCNQ1	rs2237895	C/A	0.49	1.31 (1.20–1.43)	**3.1×10^{-9}**	1.76 (1.47–2.10)	**3.7×10^{-10}**	0.98 (0.82–1.17)	0.79	**1.7×10^{-4}**
KCNQ1	rs2299620	C/T	0.55	1.34 (1.22–1.47)	**7.8×10^{-10}**	1.92 (1.60–2.31)	**4.1×10^{-12}**	0.93 (0.77–1.12)	0.47	**9.9×10^{-6}**

In the allele column, the risk allele for diabetes as observed in other studies (3–5) is given first. Frequency is the frequency of the risk allele. OR is the OR for diabetes per copy of the risk allele (without considering parent-of-origin effects). OR_M is the OR associated with a maternally derived risk allele and OR_F is the OR associated with a paternally derived risk allele. P_{dif} is the P value for the null hypothesis of no parent-of-origin effect (e.g., $OR_M = OR_F$). $P < 0.05$ shown in bold.

TABLE 3
Analysis of parent-of-origin effects in the association with quantitative metabolic traits in American Indians

Gene	SNP	*N*	Effect (95% CI)	*P*	$Effect_M$ (95% CI)	*P*	$Effect_F$ (95% CI)	*P*	P_{dif}*
Associations with percentage body fat†									
KLF14	rs4731702	351	0.98 (0.95–1.02)	0.306	0.97 (0.91–1.04)	0.391	0.99 (0.93–1.06)	0.802	0.712
MOB2	rs2334499	350	0.97 (0.94–1.01)	0.109	0.95 (0.89–1.02)	0.138	0.99 (0.93–1.06)	0.828	0.457
KCNQ1	rs231362	336	1.00 (0.93–1.07)	0.943	0.98 (0.87–1.11)	0.765	1.01 (0.91–1.13)	0.851	0.752
KCNQ1	rs2237892	400	0.98 (0.94–1.01)	0.164	1.00 (0.93–1.06)	0.924	0.95 (0.89–1.02)	0.175	0.451
KCNQ1	rs2237895	396	0.98 (0.94–1.01)	0.210	0.98 (0.92–1.05)	0.565	0.97 (0.91–1.04)	0.436	0.895
KCNQ1	rs2299620	356	0.99 (0.95–1.02)	0.510	1.01 (0.94–1.08)	0.887	0.97 (0.90–1.04)	0.417	0.577
Associations with insulin sensitivity (mg/kg estimated metabolic body size · min)‡									
KLF14	rs4731702	351	0.96 (0.92–0.99)	**0.008**	0.97 (0.91–1.03)	0.272	0.95 (0.89–1.01)	0.076	0.682
MOB2	rs2334499	350	1.02 (0.98–1.05)	0.337	1.02 (0.96–1.08)	0.542	1.01 (0.95–1.07)	0.689	0.903
KCNQ1	rs231362	336	1.03 (0.96–1.09)	0.407	1.03 (0.92–1.16)	0.585	1.02 (0.93–1.13)	0.645	0.923
KCNQ1	rs2237892	400	1.01 (0.98–1.04)	0.680	1.02 (0.96–1.08)	0.475	0.99 (0.93–1.05)	0.775	0.559
KCNQ1	rs2237895	396	1.01 (0.98–1.04)	0.541	1.02 (0.96–1.08)	0.547	1.00 (0.94–1.06)	0.952	0.749
KCNQ1	rs2299620	356	1.01 (0.98–1.04)	0.642	1.02 (0.95–1.09)	0.581	1.00 (0.93–1.06)	0.934	0.715
Associations with insulin secretion (μU/mL)§									
KLF14	rs4731702	247	0.97 (0.86–1.08)	0.566	1.04 (0.85–1.28)	0.689	0.90 (0.73–1.10)	0.299	0.387
MOB2	rs2334499	246	1.00 (0.90–1.11)	0.972	1.07 (0.88–1.30)	0.480	0.93 (0.77–1.13)	0.462	0.385
KCNQ1	rs231362	236	1.02 (0.82–1.26)	0.893	0.76 (0.53–1.08)	0.130	1.30 (0.94–1.80)	0.116	**0.046**
KCNQ1	rs2237892	286	0.86 (0.78–0.95)	**0.003**	0.77 (0.64–0.92)	**0.006**	0.96 (0.79–1.16)	0.674	0.175
KCNQ1	rs2237895	283	0.86 (0.77–0.95)	**0.004**	0.78 (0.65–0.94)	**0.011**	0.94 (0.78–1.13)	0.511	0.251
KCNQ1	rs2299620	256	0.85 (0.76–0.94)	**0.002**	0.72 (0.59–0.88)	**0.002**	0.99 (0.81–1.21)	0.928	0.078

Effect is that on trait values per copy of the risk allele, expressed as a multiplier; $effect_M$ is the effect for a maternally derived risk allele; $effect_F$ is the effect for a paternally derived risk allele. $P < 0.05$ shown in bold. *P_{dif} is the *P* value for the null hypothesis that the parental effects are equal ($effect_M$ = $effect_F$). †Association with percentage body fat with adjustment for age, sex, and heritage. ‡Association with insulin sensitivity with adjustment for age, sex, heritage, and percentage body fat. §Association with acute insulin secretion in individuals with normal glucose tolerance with adjustment for age, sex, heritage, percentage body fat, and insulin sensitivity.

different parent-specific expression profiles, but functional studies and more detailed mapping of this region are required for confirmation. In general, there was little evidence for heterogeneity in the ORs between the Icelandic and Pima Indian populations. The one exception involved the *KCNQ1* SNP rs231362, for which a protective effect of a paternally derived C allele was suggested in Pima Indians in addition to the increased risk associated with a maternally derived C allele seen in both populations. However, the risk allele was at high frequency in Pima Indians (0.90), so this finding involves a relatively small number of individuals.

The strongest associations with diabetes in the Pima Indians are with the cluster of *KCNQ1* SNPs rs2237892/rs2273895/rs2299620. These associations, which achieved genome-wide statistical significance in the current study, were "missed" in the initial genome-wide association studies in European populations, because of the high frequency of the risk alleles, and were identified in East Asian populations (3,4). In East Asian populations, the three SNPs are in moderate linkage disequilibrium and may represent independent association signals. In the Pima Indians, the linkage disequilibrium is stronger and the primary association appears to be with rs2299620; the relatively strong association between diabetes and a maternally derived risk allele at rs2299620 in Pima Indians may reflect co-occurrence of the "independent" associations from other populations on a single haplotype. In the Pima Indians, it thus is likely that the functional variants, which remain unidentified, are most strongly concordant with rs2299620.

Maternally derived alleles at rs4731702 in *KLF14* are associated with expression of a number of genes that are correlated with insulinemia and other characteristics of metabolic syndrome (20). The diabetes risk allele also was associated with hyperinsulinemia in a previous study, which suggests increased insulin resistance, although parent-of-origin effects were not assessed (1). In the current study, an association with increased insulin resistance was observed using a direct measure, but a parent-of-origin effect was not seen.

Several previous studies have suggested that *KCNQ1* SNPs influence diabetes risk through an effect on insulin secretion (3,21–27); however, many of these studies have included individuals who may have had impaired insulin secretion secondary to impaired glucose tolerance. The current study confirms this finding in individuals with normal glucose tolerance. Furthermore, because maternally derived risk alleles were associated with lower insulin secretion but paternally derived alleles were not, the current study is the first to suggest that the effects on insulin secretion are subject to imprinting. Because *KCNQ1* is imprinted in most, but not all, human tissues (28), studies of imprinting in tissues relevant to insulin secretion (pancreatic islets, central nervous system, duodenum) would be mechanistically informative.

Among ~65 variants identified as reproducibly associated with type 2 diabetes, 4 now have been robustly demonstrated to have parent-of-origin effects. This is a high proportion relative to other disorders and is more than expected given estimates that ~1% of the human genome is imprinted (29). Because many imprinted genes are related to fetal growth and development (29,30), these findings may reflect the importance of these processes in the development of type 2 diabetes. The findings of the current study also illustrate the importance of considering parent-of-origin effects when present, and they demonstrate the extent to which the contribution of individual loci to development of type 2 diabetes varies with ethnicity. In European populations, the *TCF7L2* variant rs7903146 is the

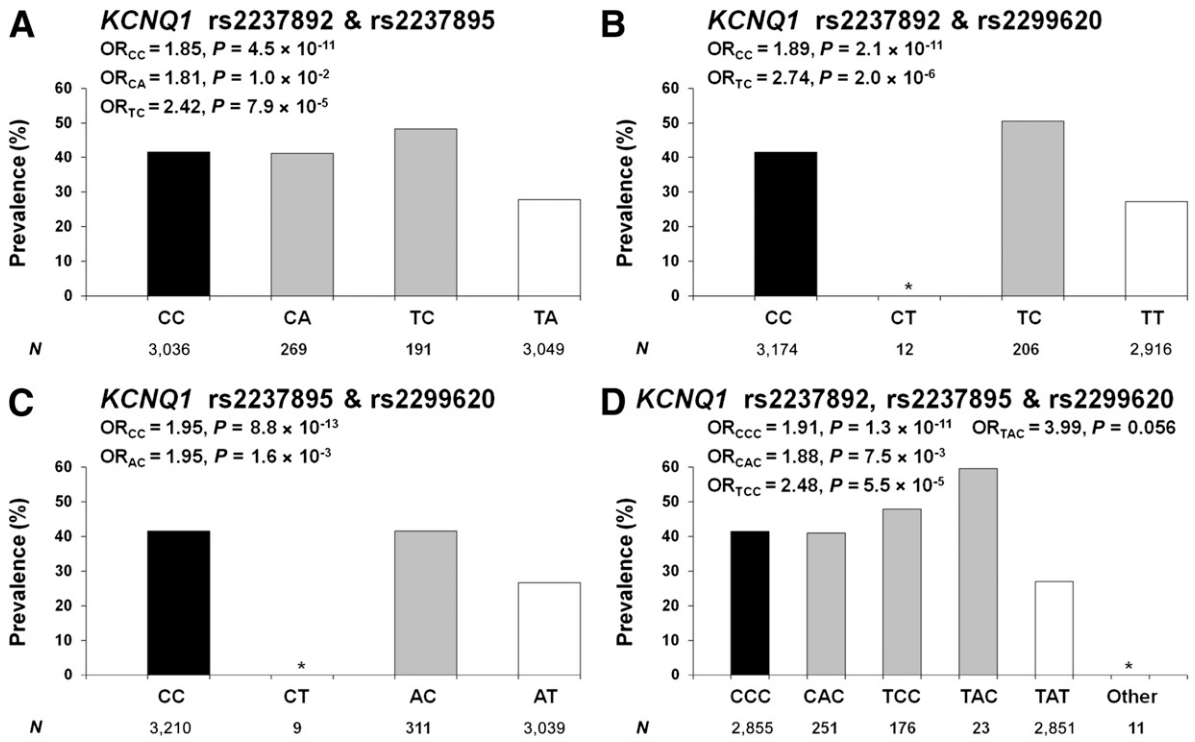

FIG. 2. Prevalence of diabetes according to the maternally derived haplotype for pairs of SNPs at *KCNQ1* and for the three SNP haplotype. *A*: Prevalence for haplotypes of rs2237892 and rs2237895. *B*: Prevalence for haplotypes of rs2237892 and rs2299620. C: Prevalence for haplotypes of rs2237895 and rs2299620. *D*: Prevalence for the three SNP haplotypes of rs2237892, rs2237895, and rs2299620. The *x*-axis shows the alleles comprising each haplotype, with the alleles listed in the same order as the markers. The OR is given comparing each haplotype containing at least one risk allele with the one containing low-risk alleles at all markers (which is shown as the open bar). *Haplotypes are too rare for reliable estimates. All results are adjusted for age, sex, birth year, heritage, and exposure to diabetes in utero. Build 37 positions for the SNPs are as follows: 2839751 (rs2237892), 2857194 (rs2237895), and 2858295 (rs2299620).

strongest known genetic contributor to diabetes risk, because it has OR of ~1.4 and accounts for ~1.3% of variation in liability for diabetes. In the Pima Indians, however, the *TCF7L2* variants have little association with type 2 diabetes (OR, 1.04; 31), and the OR of 1.34 seen in the current study for the *KCNQ1* SNP rs2299620 (without consideration of parent-of-origin effects) corresponds to ~1.6% of the variance in liability for diabetes. When the parent-of-origin effect is taken into account, there is nearly a 15% difference in prevalence of diabetes between carriers of a maternally derived C allele at rs2299620 and carriers of a maternally derived T allele. The proportion of variance in liability for diabetes that the association explains in the Pima Indians is ~4.0%. This represents one of the largest contributions reported for a single variant to type 2 diabetes risk in any human population.

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R.L.H. wrote the manuscript, researched data, and contributed to discussion. T.G., Y.L.M., J.F., W.C.K., S.K., C.B., 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.

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