Assessing the Role of 98 Established Loci for BMI in American Indians

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Objective: Meta-analyses of genome-wide association studies in Europeans have identified >98 loci for BMI. Transferability of these established associations in Pima Indians was analyzed.

Methods: Among 98 lead single nucleotide polymorphisms (SNPs), 82 had minor allele frequency ≥ 0.01 in Pima Indians and were analyzed for association with the maximum BMI in adulthood (n = 3,491) and BMI z score in childhood (n = 1,958). Common tag SNPs across 98 loci were also analyzed for additional signals.

Results: Among the lead SNPs, 13 (TMEM18, TCF7L2, MRPS33P4, PRKD1, ZFP64, FTO, TAL1, CALCR, GNPDA2, CREB1, LMX1B, ADCY9, NLRC3) were associated with BMI (P ≤ 0.05) in Pima adults. A multi-allelic genetic risk score (GRS), which summed the risk alleles for 82 lead SNPs, showed a significant trend for a positive relationship between GRS and BMI in adulthood (beta = 0.48% per risk allele; P = 1.6 × 10−9) and BMI z score in childhood (beta = 0.024 SD; P = 1.7 × 10−7). GRS was significantly associated with BMI across all age groups ≥ 5 years, except for those ≥ 50 years. The strongest association was seen in adolescence (age 14-16 years; P = 1.84 × 10−8).

Conclusions: In aggregate, European-derived lead SNPs had a notable effect on BMI in Pima Indians. Polygenic obesity in this population manifests strongly in childhood and adolescence and persists throughout much of adult life.

Introduction

Heritable factors are estimated to explain 40% to 70% of interindividual variability in BMI (1). Some genes may have a similar impact on BMI across all populations, whereas others may have ethnic-specific differences in their effects. Prior meta-analyses of genome-wide association studies (GWAS), predominately from large populations of European ancestry, have identified at least 98 loci containing single-nucleotide polymorphisms (SNPs) associated with BMI at genome-wide statistical significance (P < 5 × 10−8) (2-4). However, for the most part, it is unknown how these “established” SNPs affect BMI in isolated populations.

The Pima Indians of Arizona are a relatively isolated population with a high prevalence of obesity (5). To identify genetic variants that may contribute to obesity in this population, we previously conducted a GWAS for BMI and also directly sequenced physiologic candidate genes, such as those in the leptin-melanocortin pathway; these studies have identified both common and rare variants in MC4R, SIM1, LEPR, and MAP2K3 that associate with BMI in Pima Indians (6-10). In the current study, we analyze another category of genes, the 98 established GWAS BMI loci, by directly genotyping the lead SNP or a proxy that tags the lead SNP in a longitudinally studied population-based sample of Pima Indians. Furthermore, we also analyze tag SNPs, which capture common variation (r² ≥ 0.85 with minor allele frequency [mAF] ≥ 0.05) across these GWAS loci, in this population to identify potential additional susceptibility variants.

Methods

Subjects

Subjects were derived from a longitudinal study of the etiology of type 2 diabetes (T2D) among the Gila River Indian Community in Arizona (5), where most residents are of Pima Indian heritage. The study protocols were approved by the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases. All residents (age ≥ 5 years) of a geographical section of the community were invited to participate in outpatient biennial exams, which included measures of height and weight for calculation of BMI (kilograms divided by meters squared) as well as a 75-g oral glucose tolerance test to determine diabetes according to...
the criteria of the American Diabetes Association (11) (participants were examined from 1965 to 2007; mean ± SD exams per person, 5.2 ± 3.8; follow-up time, 19 ± 12 years). Characteristics of the 3,491 full-heritage Pima Indians (defined as eight-eighths Pima including the Tohono O’odham Nation) included in the analysis for each of the established BMI SNPs are shown in Table 1. Maximum BMI in adulthood was defined as the highest recorded BMI from an examination at age ≥ 15 years. Because diabetes duration and treatment can affect body weight, BMI was also analyzed as the highest BMI recorded at an examination at age ≥ 15 years when the subject was non-diabetic as confirmed by the oral glucose tolerance test. Individuals who did not have a measure of BMI from a non-diabetic examination were excluded from this analysis. To assess susceptibility to obesity in childhood, we analyzed the maximum age- and sex-adjusted BMI z score across examinations during childhood (between the ages of 5 and 15 years). Median BMI for Pima girls and boys (50th percentile of the Pima population) is at the 95th percentile of the US population at every age (8). Because the distribution of BMI in Pima children is very different from that in standard populations, we used a Pima-specific z score for these calculations. It was calculated by subtracting the mean from BMI and dividing by its SD in categories of age (1 year) and sex in all research participants. Given the longitudinal nature of the study, many individuals were analyzed as both children and adults. Thus, the analyses of childhood BMI z score and adulthood BMI were not independent.

Because genetic associations with BMI may vary with age, we further assessed these associations in different age groups. Twelve discrete age groups were analyzed (ages 5-7, 8-10, 11-13, 14-16, 17-19, 20-24, 25-29, 30-34, 35-39, 40-49, 50-59, and ≥ 60 years), and all individuals who were examined within a given age category were analyzed. If an individual had more than one examination in a given category, the examination closest to the midpoint of the category was used for analysis.

In the same longitudinal study, genotypic data were also available in 3,298 additional individuals whose ethnicity was not full-heritage Pima Indian (defined as seven-eighths Pima Indian or less; most of these individuals were four-eighths Pima Indian). To identify additional variants in 98 established BMI loci, SNPs associated with BMI in full-heritage Pima Indians were analyzed for replication in this “mixed-heritage” sample.

**Genotyping of lead SNPs and tag SNPs**

Lead SNPs at 30 of the previously established loci were genotyped by TaqMan Allelic Discrimination assay (Thermo Fisher Scientific, Waltham, Massachusetts). Additional data were available from prior genotyping on a custom Axiom array (Affymetrix, Santa Clara, California) designed to capture common variants (mAF ≥ 0.05 at r² ≥ 0.85 in 300-kilobase windows) across the genome, identified from whole genome sequence data of 266 full-heritage Pima Indians (12). Genotypes were identified using Analysis Suite software version 1.1.0.6161 (Affymetrix). These data were used to obtain genotypes on 52 additional lead SNPs, which included 12 lead SNPs directly genotyped on the array and 40 proxies (r² ≥ 0.85 with the lead SNP in full-heritage Pima Indians with mean ± SD r² = 0.97 ± 0.04; r² between the lead SNP and its proxy is listed in Supporting Information Table S1) on the array. Thus, 82 established variants were analyzed. Sixteen lead SNPs with mAF < 0.01 in full-heritage Pima Indians in the whole genome sequence data were not analyzed for association with BMI. Supporting Information Table S2 lists all 98 lead SNPs along with their genotyped proxies in the present study. Additional variation across the 98 loci was analyzed by using genotypic data for ~6,000 tag SNPs derived from the Axiom array. All genotypic data passed quality control metrics of genotype call rate ≥ 90%, no deviation from Hardy–Weinberg equilibrium (P ≥ 1 × 10⁻⁴), and discrepant rate ≤ 2 pairs among 100 blind duplicate pairs (12).

**Analysis of cis-acting expression quantitative trait loci in adipose tissues**

Percutaneous abdominal adipose tissue biopsies from 201 non-diabetic Pima Indians were characterized for expression using the Human Exon 1.0 ST Array microarray chips (Affymetrix), as previously described (13).

**Statistical analysis**

The association of genotype with BMI was analyzed by linear regression using a model fitted with a variance components covariance structure to account for genetic relatedness among individuals. The genetic relatedness matrix was estimated as the proportion of the genome shared identical by descent (IBD) between each pair of individuals who had been genotyped (a total of 29,648,850 pairs) (14). Shared IBD genomic segments were identified with the fastIBD function of Beagle package (15) using 482,616 autosomal markers with mAF > 0.05. Mixed models were fitted using the SOLAR package (16). The natural logarithm of BMI or the childhood BMI z score was taken as the dependent variable. Results were adjusted for age, sex, birth year, and the first five genetic principal components. As the lead SNPs were previously established as associated with BMI at genome-wide statistical significance, the α level for significant association of these lead SNPs with BMI in the present study was set at 0.05. For additional variants at each locus, analyses were conducted to identify the most strongly associated BMI variants in Pima Indians. In addition, a conditional analysis was conducted in which the European GWAS lead SNP was included as a covariate in the model to determine whether the signal additionally contributed to the association. In both conditional and unconditional analyses, variants with significant associations were further evaluated using linear regression models adjusted for age, sex, and IBD.

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**Table 1: Characteristics of full-heritage Pima Indians with longitudinal measures of BMI**

| Characteristic                                      | n     | Male, % | Age (y), mean ± SD | BMI (kg/m²), mean ± SD |
|----------------------------------------------------|-------|---------|--------------------|------------------------|
| Maximum adult BMI (any examination)                | 3,491 | 42      | 36 ± 13            | 38 ± 9                 |
| Maximum adult BMI (nondiabetic examination)        | 2,862 | 42      | 32 ± 12            | 36 ± 8                 |
| Maximum childhood BMI                               | 1,958 | 45      | 11 ± 3             | 24 ± 7                 |

Maximum adult BMI is maximum lifetime BMI recorded at age ≥ 15 years. Maximum adult BMI (nondiabetic examination) is maximum BMI recorded at a nondiabetic examination at age ≥ 15 years. Maximum childhood BMI was identified between the ages of 5 and 15 years.
### Table 2: Transferability analysis of established BMI SNPs with maximum adult BMI, maximum BMI recorded at a nondiabetic examination, and maximum childhood BMI z score in longitudinally studied full-heritage Pima Indians

| Lead SNP   | Locus (in nearest) | Allele R/N | RAF (SD) | Beta (log_10) | P     | Beta (log_10) | P     | Beta (SD) | P     | Beta (SD) | P     | I^2 (%) | P_{het} |
|------------|--------------------|------------|----------|---------------|-------|---------------|-------|------------|-------|------------|-------|---------|--------|
| rs2867125  | TMEM18             | C/T        | 0.86     | 0.13          | 0.030 | 3.0 × 10^{-4} |       | 0.035      | 6.0 × 10^{-6} | 0.131 | 0.005     |       | 0.87    | 0.06   |
| rs7903146  | TCF7L2             | C/T        | 0.92     | 0.16          | 0.039 | 4.9 × 10^{-4} |       | 0.051      | 2.0 × 10^{-6} | 0.088 | 0.18      |       | 0.75    | 0.02   |
| rs13041126 | MRPS33P4           | T/C        | 0.67     | 0.08          | 0.018 | 0.004         |       | 0.019      | 0.006        | 0.051 | 0.16      |       | 0.73    | 0.02   |
| rs1285454  | PAFX1              | C/A        | 0.78     | 0.09          | 0.020 | 0.005         |       | 0.016      | 0.04         | 0.066 | 0.11      |       | 0.63    | 0.02   |
| rs6091540  | ZFBI4              | C/T        | 0.67     | 0.07          | 0.016 | 0.01          |       | 0.017      | 0.01         | 0.033 | 0.36      |       | 0.73    | 0.02   |
| rs7193144  | FAT              | C/T        | 0.14     | 0.10          | 0.022 | 0.01          |       | 0.029      | 0.002        | 0.14  | 0.003     |       | 0.44    | 0.08   |
| rs977747  | TAL1               | T/G        | 0.74     | 0.08          | 0.017 | 0.02          |       | 0.021      | 0.004        | 0.061 | 0.13      |       | 0.47    | 0.02   |
| rs9641123  | CALCR              | C/G        | 0.09     | 0.11          | 0.023 | 0.02          |       | 0.021      | 0.005        | 0.059 | 0.32      |       | 0.39    | 0.01   |
| rs10938397 | GNPDA2             | G/A        | 0.31     | 0.06          | 0.014 | 0.02          |       | 0.014      | 0.005        | 0.095 | 0.009     |       | 0.43    | 0.03   |
| rs17203016 | CREB1              | G/A        | 0.15     | 0.08          | 0.017 | 0.03          |       | 0.016      | 0.006        | 0.073 | 0.12      |       | 0.20    | 0.02   |
| rs10736082 | LMX1B              | A/G        | 0.75     | 0.06          | 0.014 | 0.04          |       | 0.017      | 0.02         | 0.056 | 0.15      |       | 0.43    | 0.02   |
| rs2531995  | ADCY9              | T/C        | 0.18     | 0.07          | 0.016 | 0.04          |       | 0.010      | 0.02         | 0.082 | 0.06      |       | 0.59    | 0.02   |
| rs75874    | NLRC3              | T/C        | 0.11     | 0.07          | 0.018 | 0.05          |       | 0.014      | 0.01         | 0.074 | 0.15      |       | 0.27    | 0.02   |
| rs1460676  | FIGN               | C/T        | 0.07     | 0.08          | 0.018 | 0.11          |       | 0.018      | 0.016        | 0.143 | 0.03      |       | 0.22    | 0.02   |
| rs9374842  | LOC285762          | T/C        | 0.86     | 0.02          | 0.003 | 0.68          |       | 0.011      | 0.02         | 0.139 | 0.005     |       | 0.74    | 0.01   |
| rs1412645  | MIR548A2           | A/G        | 0.73     | 0.01          | 0.002 | 0.78          |       | 0.003      | 0.02         | 0.114 | 0.003     |       | 0.55    | 0.01   |
| rs1555543  | PTBP2              | C/A        | 0.54     | -0.01         | -0.001 | 0.93       |       | -0.001     | 0.89         | 0.076 | 0.03      |       | 0.57    | 0.02   |

Data given only for SNPs associated with maximum BMI in adulthood and/or maximum BMI z score in childhood with P ≤ 0.05 in full-heritage Pima Indians (n as indicated). Risk allele defined as allele with higher risk for BMI in Europeans. Beta and P values adjusted for age, sex, birth year, and the first five genetic principal components. BMI is log_{10}-transformed before analysis to approximate normal distribution. Data for meta-analysis of GIANT are derived from the public database, and beta coefficients are expressed in SD units by inverse Gaussian transformation of BMI. For comparison with beta coefficients in Europeans, maximum BMI in Pima Indians is also transformed using an inverse Gaussian transformation; results presented as beta (SD) and used in heterogeneity analyses. \( I^2 \) represents percentage of variance in effect attributable to heterogeneity between Pima Indians and Europeans, and \( P_{het} \) is P value for null hypothesis that the two beta coefficients are equal.

Bold values indicate P ≤ 0.05.

*Proxy SNP used for genotyping (listed in Supporting Information Table S1).

N, nonrisk allele; R, risk allele; RAF, risk allele frequency; A, T, C, G, nucleotide.
nominal associations ($P < 0.05$) in full-heritage Pima Indians were assessed for replication in the mixed-heritage individuals. We report all SNPs with nominal evidence for replication in these mixed-heritage individuals ($P < 0.05$). We also note whether any of the new signals for BMI in the combined analysis achieved significance after a Bonferroni correction for the 6,000 variants tested ($P < 8.3 \times 10^{-6}$).

To analyze the effects of all 82 established BMI SNPs in aggregate, we constructed a multi-allelic genetic risk score (GRS). For variants genotyped as part of the Axiom array, missing data for these analyses were imputed with IMPUTE2 using whole genome sequence data of 266 full-heritage Pima Indians (17). For variants genotyped separately, missing data were inferred from genotypes of relatives using a likelihood-based method implemented in MLINK, as previously described (18). The GRS was computed for each subject as the sum of the number of risk alleles observed at each locus (i.e., all variants were given equal weight). The effect size of a 1-unit difference in the GRS represents the effect per risk allele at any of the 82 loci. We also conducted analyses in which the GRS was calculated for each subject as the sum of the number of risk alleles observed at each locus weighted by the published effect size in Europeans (taken from the Genetic Investigation of Anthropometric Traits [GIANT], https://portals.broadinstitute.org/collaboration/giant), divided by the sum of the weights. The results of these analyses (shown in Supporting Information Figure S1) were similar to those with equally weighted effects. Hence, we report the results for the GRS with equal weight for each locus.

To test whether the effects in Pima Indians were consistent with those in Europeans, we compared the regression coefficients observed in Pima Indians with those obtained in GIANT. For these analyses, BMI associations in Pima Indians were analyzed using the inverse Gaussian transformation of the rank of maximum BMI in adulthood to express beta coefficients and SEs in SD units comparable with those used in GIANT. Beta coefficients were compared by the Cochran $Q$ test of homogeneity, and heterogeneity was quantified by the $I^2$ statistic (19). To test for heterogeneity across all 82 lead SNPs analyzed, a combined $Z^*$ score was calculated by combining $P$ values for the null hypothesis of homogeneity across all 82 markers, using Stouffer’s method, as previously.
described (18-20). As constructed here, if \( Z^* \) is positive, it indicates that beta coefficients are generally stronger in Pima Indians than in Europeans, whereas if \( Z^* \) is negative, it indicates that beta coefficients, on average, are weaker in Pima Indians.

To analyze diabetes in the mixed model, a binary linear model was used in which this discrete trait was treated as a continuous (0,1) variable, and these analyses were used for calculating the \( P \) value for association.

The odds ratio (OR) was calculated following the method of Haggstrom from the regression coefficient (\( b \)) and the residual variance (\( \sigma^2 \)) in the binary linear model (i.e., \( \ln[\text{OR}] = b/\sigma^2 \)) (21).

Results

Transferability analysis in Pima Indians of 98 lead SNPs previously shown to associate with BMI in Europeans

Sixteen of the ninety-eight lead SNPs that were associated with BMI in a meta-analysis of Europeans (Supporting Information Table S2) had mAF <0.01 in Pima Indians; therefore, these 16 SNPs were omitted from the statistical analyses. The remaining 82 lead SNPs were genotyped in full-heritage Pima Indians and analyzed for association with the maximum BMI recorded in adulthood (\( n = 3,491 \)), the maximum BMI recorded in adulthood when the subject did not have T2D (\( n = 2,826 \)), and the maximum BMI \( z \) score in childhood (\( n = 1,958 \)) (Supporting Information Table S2). Table 2 shows the SNPs associated with maximum BMI in adulthood and/or maximum BMI \( z \) score in childhood with \( P \leq 0.05 \) in full-heritage Pima Indians in which the direction of the association was consistent with that observed in Europeans. Thirteen of these lead SNPs associated with maximum BMI in adulthood included those in or near \( \text{TMEM18} \), \( \text{TCF7L2} \), \( \text{MRPS33P4} \), \( \text{PRKD1} \), \( \text{ZFP64} \), \( \text{FTO} \), \( \text{TAL1} \), \( \text{CALCR} \), \( \text{GNPDA2} \), \( \text{CREB1} \), \( \text{LMX1B} \), \( \text{ADCY9} \), and \( \text{NLRC3} \) (\( P \leq 0.05 \); Table 2). SNPs with the strongest association with BMI in adulthood were rs2867125 in \( \text{TMEM18} \) (\( P = 3.0 \times 10^{-4} \), beta = 0.03 on loge scale, which corresponds to a 3.0% increase in BMI per copy of the risk allele) and rs7903146 in \( \text{TCF7L2} \) (\( P = 4.9 \times 10^{-4} \), beta = 0.04 or a 4.0% increase in BMI). Most of these SNPs were also associated with maximum BMI at a nondiabetic examination in adulthood (\( P \leq 0.05 \); Table 2) and maximum BMI \( z \) score in childhood (\( P \leq 0.05 \); Table 2) in the Pima Indian sample, whereas the SNP with the strongest association with BMI \( z \) score in childhood was rs7193144 in \( \text{FTO} \) (\( P = 0.003 \), beta = 0.14 SD units per copy of the risk allele).
### TABLE 3 Associations of additional SNPs with BMI in 98 established BMI loci in an expanded population-based study of American Indians

| Locus | RS   | SNP   | Freq allele 1, full-heritage (n = 3,491) | Freq allele 1, mixed-heritage (n = 3,298) | Freq allele 1, combined (n = 6,789) | BMI in GIANT (n = 339,224) |
|-------|------|-------|---------------------------------------|----------------------------------------|-----------------------------------|-----------------------------|
|       |      |       | Maximum BMI, full-heritage              | Maximum BMI, mixed-heritage             | Maximum BMI, combined             |                             |
|       |      |       | (n = 3,491)                            | (n = 3,298)                             | (n = 6,789)                       |                             |
|       |      |       | Beta (log_{10})                         | Beta (log_{10})                         | Beta (log_{10})                   | Beta (log_{10})             |
|       |      |       | P                                      | P                                      | P                                 | P                           |
|       |      |       | Beta (log_{10})                         | P                                      | Beta (log_{10})                   | P                           |
|       |      |       | Beta (log_{10})                         | P                                      | Beta (log_{10})                   | P                           |

- **NLRC3** rs3751837 T/C 0.11 0.22 0.023 0.02 0.028 0.002 0.028 5.8 × 10^{-5} - -
- **NLRC3** rs1664156 C/G 0.06 0.03 0.025 0.05 0.025 0.04 0.029 0.001 0.028 0.003
- **NLRC3** rs758747 T/C 0.13 0.27 0.018 0.05 0.019 0.03 0.019 0.004 0.023 1.5 × 10^{-10}
- **PGEP1** rs12462812 A/G 0.19 0.01 0.016 0.03 0.022 0.007 0.019 0.0005 - -
- **ADCY9** rs2531982 A/G 0.86 0.70 0.023 0.01 0.022 0.01 0.022 0.0005 -0.20 5.0 × 10^{-7}
- **ADCY9** rs2531995 T/C 0.21 0.59 0.016 0.04 0.017 0.02 0.017 0.002 0.024 7.6 × 10^{-10}
- **ADCY9** rs35384844 T/C 0.86 0.95 0.017 0.04 0.020 0.02 0.018 0.004 - -
- **CADM1** rs149906922 A/T 0.38 0 0.019 0.001 0.014 0.03 0.016 0.0007 - -
- **CADM1** rs718484 A/G 0.28 0.09 0.019 0.003 0.020 0.005 0.016 0.001 0.007 0.29
- **CADM1** rs77550241 A/G 0.25 0.01 0.018 0.008 0.020 0.007 0.015 0.003 - -
- **CADM1** rs17183432 T/C 0.78 1 0.020 0.005 0.017 0.03 0.014 0.007 - -
- **CADM1** rs11215485 T/C 0.30 0.12 0.019 0.003 0.013 0.05 0.013 0.008 -0.001 0.85
- **CADM1** rs72306674 -/TCTC 0.25 0.01 0.016 0.01 0.017 0.02 0.013 0.01 - -
- **LRP1B** rs17602834 T/C 0.60 0.48 -0.013 0.04 -0.015 0.02 -0.015 0.0009 0.007 0.10
- **LRP1B** rs17569555 A/G 0.91 0.99 0.023 0.02 0.022 0.03 0.021 0.005 0.012 0.32
- **ZFP64** rs139447701 A/C 0.18 0 -0.019 0.008 -0.016 0.05 -0.019 0.001 - -
- **ZFP64** rs6021702 T/C 0.17 0.37 0.022 0.02 0.016 0.04 0.017 0.005 -0.003 0.56
- **ZFP64** rs11086366 A/C 0.54 0.88 0.012 0.05 0.016 0.01 0.013 0.005 0.001 0.88
- **FTO** rs1861869 C/G 0.70 0.47 -0.013 0.04 -0.015 0.02 -0.015 0.001 -0.30 9.9 × 10^{-17}
- **FTO** rs3751814 A/G 0.15 0.41 0.021 0.009 0.017 0.05 0.019 0.002 - -
- **FTO** rs7206790 C/G 0.81 0.49 -0.019 0.01 -0.016 0.04 -0.017 0.002 -0.066 3 × 10^{-10}
- **TCF7L2** rs146479796 T/C 0.80 1 -0.017 0.02 -0.017 0.03 -0.017 0.002 - -
- **TCF7L2** rs7895657 A/G 0.29 0.30 0.013 0.05 0.013 0.05 0.012 0.01 - -
- **ZBTB10** rs183925020 A/C 0.15 0.02 0.009 0.02 0.021 0.04 0.021 0.002 - -
- **ZBTB10** rs574542 A/G 0.75 0.87 0.020 0.003 0.014 0.05 0.015 0.004 0.000 0.95
- **RAR6** rs186124687 C/G 0.75 0.87 -0.016 0.01 -0.015 0.04 -0.016 0.002 - -
- **CLIP1** rs34383139 A/C 1 0 0.019 0.02 0.021 0.04 0.021 0.002 - -
- **LMI1B** rs3814120 A/G 0.30 0.07 0.015 0.02 0.014 0.03 0.015 0.002 0.022 0.0008
- **LMI1B** rs1073682 A/G 0.75 0.43 0.014 0.04 0.019 0.01 0.016 0.003 0.019 2.5 × 10^{-10}
- **LMI1B** rs16929203 T/C 0.32 0.08 0.016 0.01 0.013 0.05 0.014 0.004 - -
- **LMI1B** rs28687510 T/G 0.32 0.11 0.015 0.01 0.013 0.05 0.014 0.004 - -
- **LMI1B** rs10739682 T/C 0.89 0.63 0.023 0.03 0.018 0.05 0.018 0.01 - -
TABLE 3. (continued).

| Locus          | SNP             | Frequencies | Allele 1/2  | Beta (loge P) | P     | Beta (SD) | P     |
|----------------|-----------------|-------------|-------------|---------------|-------|-----------|-------|
| LOC342496      | rs33034270       | T/C         | 0.12        | 0.018         | 0.03  | 0.003     | 0.01  |
|                | rs12286929       | T/C         | 0.16        | 0.098         | 0.03  | 0.004     | 0.02  |
|                | rs1943217        | T/G         | 0.47        | 0.066         | 0.03  | 0.006     | 0.02  |
|                | rs12886730       | T/C         | 0.49        | 0.086         | 0.02  | 0.002     | 0.01  |
|                | rs147306320      | T/C         | 0.08        | 0.008         | 0.03  | 0.002     | 0.01  |

Data given for analysis of full-heritage Pima Indians, mixed-heritage Pima Indians, and combined samples (as indicated). Allele 1 defined as reference allele; allele 2 defined as altered allele. Beta and P values adjusted for age, sex, birth year, and the first five genetic principal components. SNPs not available in meta-analysis of GIANT are denoted with “-”.

Aggregate analysis of lead SNPs previously shown to associate with BMI in Europeans

To assess whether the European-derived risk alleles contribute in aggregate to obesity in Pima Indians, a multi-allelic GRS was created by summing the number of the risk alleles of all 82 SNPs with mAF ≥ 0.01, with equal weight for each locus. The GRS showed a significant trend for the relationship between increasing number of risk alleles in Pima Indians and maximum BMI in adulthood (Figure 1; beta = 0.0048 on loge scale, which corresponds to a 0.48% increase in BMI per unit increase of the GRS; P = 1.6 × 10\(^{-3}\)), maximum BMI at a nondiabetic examination in adulthood (beta = 0.0054 or a 0.54% increase per unit increase of the GRS; P = 2.8 × 10\(^{-16}\)), and maximum BMI \(z\) score in childhood (beta = 0.024 SD units per unit increase of the GRS; \(P = 1.7 \times 10^{-3}\)). The GRS was also strongly associated with BMI in 3,298 mixed-heritage Pima Indians (Supporting Information Figure S2). Despite BMI being a risk factor for development of T2D, the GRS for BMI was not associated with diabetes status (Figure 1; OR\(_{\text{Pima vs. European}}\) = 0.995-1.028; \(P = 0.17\)). When analyzed individually, four SNPs, rs719344 in FTO, rs12286929 in CADMI, rs1441264 in MIR548A2, and rs10938397 in GNPDA2, were nominally associated with T2D in 3,747 Pima Indians (OR = 1.21-1.15 per risk allele for BMI; \(P = 0.008-0.05\), Supporting Information Table S3), with the BMI risk allele associated with higher risk for diabetes.

Aggregate effect of 82 lead SNPs on BMI at various ages was also assessed in analyses of the longitudinal measures for BMI in Pima children and adults. The GRS was associated with higher BMI in most age groups, except for those >50 years old, and effects tended to be stronger at younger ages. The strongest effect, in terms of the regression coefficient, was observed in those 14 to 16 years old (beta = 0.0076, corresponding to a 0.76% increase in BMI per unit increase of the GRS; \(P = 1.2 \times 10^{-3}\)) (Figure 2).

Additional variants in established BMI loci associate with BMI in American Indians

To determine whether additional variants in these 98 loci associate with BMI in Pima Indians in a stronger fashion than the lead SNPs, the genotypes previously generated for ~6,000 SNPs that tag \(r^2 ≥ 0.85\) common variation (mAF ≥ 0.05) across all 98 loci were analyzed for BMI associations in full-heritage Pima Indians. Those SNPs with \(P < 0.05\) were further analyzed in the replication sample of 3,298 non–full-heritage Pima Indians from the same longitudinal study. Thirty-eight tag SNPs, which map to 18 loci, had nominal associations with BMI (\(P < 0.05\)) in both full-heritage and non–full-heritage Pima Indians (Table 3). The strongest signal for BMI all 82 lead SNPs that were analyzed. Six SNPs (in TMEM18, TCF7L2, MRPS3P4, PRKD1, TAL2, CALCR) had significant heterogeneity between the ethnic groups in which the effect of genotype on BMI was greater in Pima Indians compared with Europeans (Table 2), whereas three SNPs (in C9orf93, RASA2, SH2B1) had significant heterogeneity in which the effect was weaker in Pima Indians than in Europeans (Supporting Information Table S2). The largest difference in effect size was observed at rs903146 (TCF7L2) (beta: Pima vs. European, 0.165 vs. 0.024 BMI SD units; \(I^2 = 87.6\%; P = 0.004\)). The overall test for heterogeneity across all 82 SNPs was not statistically significant (\(Z^* = 1.21\); \(P = 0.23\)), and this result was not materially different when restricted to the 42 lead SNPs that were directly genotyped (\(Z^* = 1.84\); \(P = 0.07\)).
in adulthood was rs3751837 in NLRC3 (\(P = 5.8 \times 10^{-5}\) in the combined analysis of full-heritage and non–full-heritage Pima Indians), whereas SNPs rs12462812 in PGPEP1, rs2531982 in ADCY9, rs149906922 in CADM1, and rs17602834 in LRP1B had associations with \(P < 10^{-3}\). In addition, to determine whether additional variants contribute to BMI, independent of the lead SNP, those SNPs with \(P < 0.05\) after conditioning on the established lead SNPs were replicated in non–full-heritage Pima Indians. Nine tag SNPs in seven loci showed BMI associations in both samples (\(P < 0.05\) after conditioning on the established SNP; Table 4). For five of these seven loci (PGPEP1, CADM1, CLIP1, LRP1B, EHB1), the previously established SNP were not significantly associated with BMI in Pima Indians, whereas for ZFP64 and TCF7L2, there was evidence of both the established SNP and new SNP affecting BMI. The strongest potential new signals for BMI in adulthood were rs6021702 in ZFP64 (\(P = 8.4 \times 10^{-4}\) after conditioning on the lead SNP rs6091540) and rs12462812 in PGPEP1 (\(P = 8.8 \times 10^{-4}\) after conditioning on the lead SNP rs17724992). However, no variant, in either the unconditional or conditional analyses, achieved statistical significance after correction for testing \(\sim 6,000\) SNPs (\(P < 8.3 \times 10^{-4}\)).

### Identification of cis-acting expression quantitative trait loci established or putative BMI-associated variants

To determine whether any of the 82 previously established or 9 newly identified putative SNPs associated with BMI may function as a cis-acting expression quantitative trait locus (cis-eQTL), genotypic data were merged with expression data from adipose tissue biopsies collected from 201 Pima Indians. Four established lead SNPs (rs2531995 in ADCY9, rs2176598 in HSD17B12, rs657452 in AGBL4, rs10150332 in NRXN3) correlated with RNA levels of the respective gene with \(P < 0.05\) (Table 5). The strongest correlation was observed between the 3′-UTR (untranslated region) SNP rs2531995 in ADCY9 and its expression (\(P = 5.3 \times 10^{-6}\)), whereas the allele for higher BMI in Europeans (T at rs2531995) was associated with reduced ADCY9 expression in adipose tissue. The established intronic SNP rs2176598 in HSD17B12 also had evidence of being a cis-eQTL; it correlated with HSD17B12 expression in adipose tissue (\(P = 0.006\)), in which the BMI risk allele T had a lower RNA level in Pima Indians. This correlation was replicated in 298 adipose tissues reported in the GTEx (Genotype-Tissue Expression) database (GTEx Analysis Release V6p; https://gtexportal.org/home/; \(P = 2.2 \times 10^{-35}\)) in a direction consistent with that in Pima Indians.

### Discussion

Recent meta-analyses of GWAS have identified many genetic variants that associate with BMI across multiple studies of European populations (2-4); however, the extent to which these variants contribute to the high rate of obesity found in more isolated populations is not well understood. Therefore, in the current study, we determined the effect of 98 established SNPs on BMI measured in Pima Indians of Arizona. Our analysis showed that 13 established SNPs were associated with maximum BMI in adulthood and 7 SNPs were associated with maximum BMI z score in childhood with \(P \leq 0.05\), with the direction of the association consistent with that observed in Europeans. The \(P\) values for the associations between SNPs and BMI were much more significant among the 339,224 Caucasians compared with the 3,491 Pima Indians; no SNP in the Pima Indian analysis achieved genome-wide statistical significance. This is not surprising given the relatively small sample

#### Table 4 Additional SNPs independent of lead SNPs in 7 of 98 established BMI loci associated with BMI in expanded population-based study of American Indians

| Locus | SNP | Freq | Allele 1 |BI M | Allele 2 | European | Lead SNP | Beta (loge) | Beta (SD) | P after conditioning for lead SNP | P after conditional analysis for lead SNP |
|-------|-----|------|----------|-----|----------|----------|----------|------------|----------|-----------------------------------|------------------------------------------|
| ZFP64 | rs6021702 | 0.17 | T/C | 0.76 | C/T | 0.24 | 0.024 | 0.02 | 0.003 | 0.56 |
| P5BP1 | rs12462812 | 0.19 | A/G | 0.76 | C/T | 0.24 | 0.024 | 0.02 | 0.003 | 0.56 |
| P5BP1 | rs146479796 | 0.80 | A/G | 0.76 | C/T | 0.24 | 0.024 | 0.02 | 0.003 | 0.56 |
| CADM1 | rs718484 | 0.28 | A/G | 0.76 | C/T | 0.24 | 0.024 | 0.02 | 0.003 | 0.56 |
| CADM1 | rs72306674 | 0.25 | A/G | 0.76 | C/T | 0.24 | 0.024 | 0.02 | 0.003 | 0.56 |
| CADM1 | rs72306674 | 0.25 | A/G | 0.76 | C/T | 0.24 | 0.024 | 0.02 | 0.003 | 0.56 |
| CLIP1 | rs147306320 | 0.08 | A/G | 0.76 | C/T | 0.24 | 0.024 | 0.02 | 0.003 | 0.56 |

Data given for analysis of full-heritage Pima Indians, mixed-heritage Pima Indians, and combined samples (\(n\) as indicated). Allele 1 defined as reference allele; allele 2 defined as altered allele. Conditional analysis conducted after correcting for age, sex, year, and the first five genetic principal components.
size of Pima Indians. Nonetheless, some of the SNPs had significantly stronger effects in Pima Indians than in Europeans. For example, the lead SNP rs2867125 in TMEM18 had a twofold larger effect size (beta per copy of risk allele) in Pima Indians compared with Europeans (0.13 and 0.06 SD units, respectively; P = 0.04 for difference in effect size), whereas the lead SNP rs7903146 in TCF7L2 had the largest difference in effect size between Pima Indians and Europeans (0.165 and 0.024 SD units, respectively; P = 0.005). Some variants, on the other hand, had significantly weaker effects in Pima Indians (in C9orf93, RASA2, and HSD17B12). Overall, however, the differences in effect sizes between Pima Indians and Europeans were not statistically different; this suggests that, in general, these established obesity variants have similar effects in both populations. Some of the variants analyzed in the present study were proxies that tag the lead SNPs identified in Europeans, and incomplete concordance between the tag and lead SNP could introduce heterogeneity. However, the overall test for heterogeneity was still not statistically significant when restricted to the 42 lead SNPs that were directly genotyped. Although most BMI-associated variants identified in Pima Indians are also associated with BMI in other populations, there are some ethnic-specific associations. In the current study, we did not assess variants that were primarily identified in studies of non-European ethnic groups (22).

Our longitudinal data with measures of BMI across multiple ages, spanning both childhood and adulthood, allow for a comprehensive assessment of the effect of established BMI loci on obesity risk. For example, when all 82 SNPs with mAF ≥ 0.01 were considered in aggregate, the GRS was statistically significant in relation to maximum BMI during adulthood as well as childhood in Pima Indians. In general, the effects, in terms of the strength of the (logarithmic) regression coefficient, were stronger in childhood and adolescence than in adulthood. When analyzed individually, SNPs in FTO, MC4R, GNPDA2, TMEM18, SEC16B, FAIM2, TFAP2B, TNNI3K, and LMX1B were associated with childhood obesity in a meta-analysis of 33 studies including 47,541 children predominately of European ancestry (23). We previously assessed 36 Pima-specific BMI SNPs for their role in BMI gain during lifetime and also found stronger genetic effects in childhood than in adulthood (24). In the present study, the GRS derived from established obesity variants was significantly associated with BMI in Pima Indians in most age groups, except for the oldest individuals. The strongest effects were observed in adolescence (age 14-16 years). This suggests that obesity conferred by these loci is established in childhood and adolescence in this population and persists throughout adulthood, with some attenuation at older ages.

Although obesity is a major risk factor for development of T2D, the lead BMI SNPs showed little or no evidence for association with T2D when analyzed individually or as a GRS in 3,747 Pima Indians. Statistical power for individual variants in the present study is limited because of a relatively small sample size. However, the effect of the GRS on T2D was very modest and not statistically significant, and this suggests that, on average, the effect of these established obesity loci on T2D is small.

The current study included a comprehensive assessment of the 98 established loci for new or additional signals for BMI in Pima Indians. Thirty-eight tag SNPs, which map to 18 loci, had nominal associations with BMI in both full-heritage and non–full-heritage Pima Indians (P ≤ 0.05). The strongest signal for BMI was rs3751837 in NLRC3 (P = 5.8 × 10^{-5}). We identified additional independent signals in nine tag SNPs that map to seven loci and that had slightly stronger associations with BMI than the previously reported lead SNP. Two SNPs (rs6021702 in ZFP64 and rs12462812 in PGPEP1) that were distinct from the lead SNPs provided the strongest evidence for a new independent signal for BMI. The previously established SNP in ZFP64 was significantly associated with BMI, whereas the established SNP in PGPEP1 was not associated with BMI in the Pima Indian sample. Given that causal variants at most of these loci have not been identified, different signals between Pima Indians and Europeans might be expected because of differences in linkage disequilibrium. Nevertheless, none of these additional signals achieved statistical significance after correction for testing ~6,000 SNPs (P < 8.3 × 10^{-6}).

Determining the function of SNPs identified in large meta-analyses can be challenging. Our finding that the 3’-UTR SNP rs2531995 in ADCY9 was significantly associated with reduced ADCY9 expression (P = 5.3 × 10^{-6}) in 201 adipose biopsies of Pima Indians indicates that this SNP functions as (or tags) a cis-eQTL. Although our evidence that the intronic SNP rs2176598 in HSD17B12 expression in adipose biopsies from Pima Indians was somewhat weaker (P = 0.006), this finding was supported by data provided by the GTEx consortium, in which the risk allele T at rs2176598 had a reduced HSD17B12 expression level in adipose tissues (GTEx Analysis Release V6p; P = 2.2 × 10^{-35}). ADCY9 is part of the signaling pathway of ADRB2 (25,26), whereas HSD17B12 gene expression is dysregulated in BDNF knockout mice (27). Our results suggest that these variants may affect obesity through an effect on expression of ADCY9 and HSD17B12.

In conclusion, although many individual SNPs associated with BMI in large European studies do not show significant evidence for association in the smaller Pima Indian sample, calculation of a GRS to analyze the SNPs in aggregate shows strong a association between the number of risk alleles and BMI in Pima Indian adults and children. This suggests that obesity loci identified in Europeans generally also affect BMI in Pima Indians.

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**TABLE 5 Analysis of cis-eQTL variants in established BMI loci in adipose tissue biopsies of 201 Pima Indians**

| SNP       | Gene   | Allele (R/N) | RAF | Location | Mean (R/R) | Mean (R/N) | Mean (N/N) | Beta     | P        |
|-----------|--------|--------------|-----|----------|------------|------------|------------|----------|----------|
| rs2531995 | ADCY9  | T/C          | 0.18| 3’UTR    | −1.30      | −0.25      | 0.21       | −0.24    | 5.3 × 10^{-6} |
| rs2176598 | HSD17B12 | T/C        | 0.52| Introns  | −0.28      | 0.018      | 0.29       | −0.29    | 0.006    |
| rs657452  | AGBL4  | A/G          | 0.39| Introns  | −0.34      | −0.04      | 0.18       | −0.26    | 0.007    |
| rs10150332| NRXN3  | C/T          | 0.36| Introns  | 0.13       | 0.16       | −0.22      | 0.24     | 0.02     |

Adipose tissue gene expression levels determined using Human Exon 1.0 ST Array microarray chips (Affymetrix) and expressed as batch- and sex-standardized values (SD units). Risk allele defined as allele with higher risk for BMI in Europeans. Beta and P values adjusted for age at time of biopsy and the first genetic principal component. N, nonrisk allele; R, risk allele; RAF, risk allele frequency; UTR, untranslated region.
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