Genome-Wide Linkage Analysis Assessing Parent-of-Origin Effects in the Inheritance of Type 2 Diabetes and BMI in Pima Indians

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We examined the hypothesis that imprinted genes may affect the propensity to type 2 diabetes and obesity in Pima Indians. Multipoint variance component methods were used to assess linkage of BMI (kg/m^2) and age-adjusted diabetes to loci derived from either father (LOD_{FA}) or mother (LOD_{MO}) in a genome-wide scan. Tentative evidence of loci where imprinted genes might be acting was found for diabetes with maternally derived alleles on chromosomes 5 (LOD_{MO} = 1.5) and 14 (LOD_{MO} = 1.6). Evidence of linkage of BMI to maternally derived alleles was found on chromosome 5 (LOD_{MO} = 1.7) and to paternally derived alleles on chromosome 10p (LOD_{FA} = 1.7). Additional analyses of sibling pairs who were affected by diabetes and younger than 25 years of age showed an increase of sharing of maternally derived alleles on chromosome 6 (LOD_{MO} = 3.0). We also examined sites of a priori interest where action of imprinted genes has been proposed in diabetes or obesity. We found no evidence of parent-specific linkage (of either diabetes or BMI) on chromosome 11p, a region that contains several imprinted genes, but observed weak evidence of linkage of diabetes to paternally derived alleles (LOD_{FA} = 0.9) in the region of chromosome 6q, believed to contain an exclusively paternally expressed gene or genes that cause transient neonatal diabetes mellitus. In conclusion, we determined regions of interest on chromosomes 5, 6, and 10 where imprinted genes might be affecting the risk of type 2 diabetes or obesity in Pima Indians. *Diabetes* 50: 2850–2857, 2001

The causes of type 2 diabetes and obesity are unknown in the majority of cases but are believed to reflect a mixture of genetic and environmental factors. Several pieces of evidence suggest that it may be important to consider influences of genomic imprinting—differential expression of genes depending on parent of origin. The strongest evidence for imprinting effects comes from syndromic causes of obesity and diabetes. Prader-Willi syndrome is one of the best characterized conditions arising from imprinted genes and involves loss of function of exclusively paternally expressed genes on chromosome 15q11–q12 and a phenotype that includes hyperphagia, obesity, and, in some cases, diabetes (OMIM 176270). Abnormalities of imprinted genes are also directly implicated in abnormal glucose homeostasis in transient neonatal diabetes mellitus (TNDM). This rare disorder is characterized by hyperglycemia in newborn infants, some of whom, after a period of remission, develop a syndrome similar to type 2 diabetes in adult life (OMIM 601410). Although the precise genetic cause of TNDM is not known, it is associated with duplication of a specific region of chromosome 6 (6q24.1–24.3) but only if the duplicated region is of paternal origin (1). The syndrome thus seems to involve an imprinted gene, with overexpression of a paternally expressed gene acting as a putative mechanism (1).

Recently, type 2 diabetes was reported to be associated with a genetic polymorphism on chromosome 11p close to the insulin gene (INS VNTR III), but only if the associated allele was transmitted from the father (2). The authors speculated that an imprinted gene in this region might increase the risk of type 2 diabetes (2). Imprinting effects have also been postulated in the association of this marker to type 1 diabetes (3). Finally, the association of low birth weight with later development of type 2 diabetes (4) lends some support to the hypothesis that imprinted genes might be acting in type 2 diabetes. The majority of imprinted genes seem to influence pathways that involve fetal growth or placental development, notably the gene for *IGF-2* (paternally expressed), which is also found on chromosome 11p in humans (5,6). If, as was proposed by Hattersley et al. (7), low birth weight is associated with later type 2 diabetes secondary to genetic as well as environmental effects, then this association might arise secondary to actions of imprinted genes on both fetal growth and glucose homeostasis.

Among Pima Indians, both low and high birth weight have been associated with later risk of type 2 diabetes (8). More recently, we showed an association of low birth weight to paternal but not maternal diabetes (9), consistent with the hypothesis that imprinted genes, with paternally expressed alleles, might explain the relationship.

We previously reported results of a genome-wide linkage analysis of diabetes and obesity in Pima Indians using analytic methods that did not distinguish parent-of-origin effects (10). To examine the hypothesis that imprinted genes may affect propensity to diabetes and obesity, we

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IBD, identical by descent; LOD, logarithm of odds; TNDM, transient neonatal diabetes mellitus.

now report an analysis that incorporates parent-of-origin effects in a subset of these data informative for those analyses.

RESEARCH DESIGN AND METHODS

Participants and phenotypes. The subjects of this report also are participants in the National Institutes of Health survey of diabetes in the Gila River Indian community. All members of the community who are older than 5 years of age are invited to a biennial examination that includes a 75-g oral glucose tolerance test. Diabetes is diagnosed if fasting blood glucose is ≥140 mg/dl, glucose 2 h after the glucose load is ≥200 mg/dl, or if diabetes is independently diagnosed in a clinical setting. Height and weight for calculation of BMI (kg/m^2) are measured with the participant wearing light clothing and no shoes. In this analysis, the maximum BMI observed in the longitudinal study for each individual at 15 years of age was used as a measure of the susceptibility to obesity, in keeping with the previous non–parent-specific genome-wide scan (10), after adjustment for age (as quadratic and linear terms) and sex by linear regression.

In linkage analysis, we examined the propensity to diabetes expressed as a diabetes score. This score can be analyzed as a quantitative trait and allows adjustment for different ages of onset of diabetes and for different ages at last examination in those without diabetes (11). In brief, the sex-specific cumulative incidence of diabetes as a function of age (CI_a) in the Pima population was first calculated. When diabetes was present, the diabetes score was derived as $1 - CI_a$ at the age of diagnosis of diabetes. When diabetes was not present, the score was derived as $-CI_a$ at the time of the last examination. The score thus contains information on both whether an individual developed diabetes and the age of onset of diabetes, being positive if diabetes was ever present and greater if diabetes developed at an earlier age. Conversely, a negative score was calculated when the individual was not diabetic at the last examination and was lowest in those who are known to have remained nondiabetic into old age.

Genotyping. As previously reported (10), a sample of 1,338 individuals who had participated in the longitudinal study were selected for a genomic scan for loci linked to type 2 diabetes and obesity. Criteria for inclusion were availability of DNA and membership in a nuclear family informative for diabetes (families with ≥1 sibling pair and ≥1 affected sibling) or the metabolic predictors of diabetes (families with ≥1 sibling pair with measures of insulin sensitivity and resistance [12]). These individuals consisted of 332 nuclear families and 112 extended pedigrees.

Genotypic data used were identical to those in the genomic scan reported previously (10). In brief, 503 autosomal microsatellite markers were typed in the laboratory of J. Weber, at the Marshfield Medical Research Foundation (13). An additional 13 markers were typed at Glaxo-Wellcome. The median rate of agreement between duplicate samples was 97%, and no marker had an agreement rate <90%. As previously described, the pattern of mendelian incompatibilities across all markers was used to exclude mis-specified pedigree structure (10). Possible genotyping errors were excluded, and genetic distances between markers were determined for the Pima data (10).

Linkage analysis. The variance components method of Amos (14) is a widely used nonparametric method for linkage analysis of quantitative traits. It acts on the principle that if a genetic marker is closely linked to a locus influencing a trait, then pairs of relatives who share a larger proportion of alleles identical by descent (IBD) will tend to have more similar trait values than are seen in pairs of relatives who share fewer alleles IBD. We conducted linkage analysis by modification of this method (15), partitioning IBD in siblings into proportions reflecting alleles inherited from either mother or father, thus allowing detection of potential imprinting effects.

For nonimprinted effects, variance was partitioned into *1*) an additive monogenic component linked to the region of interest (σ^2_Q), *2*) a "polygenic" component that incorporates overall familial effects (σ^2_G), and *3*) an "environmental" component that incorporates effects unique to the individual (σ^2_E), as detailed by Amos (14). Under the assumption of no recombination between the trait and marker loci, the phenotypic variance-covariance matrix (Ω) for individuals in a pedigree is as follows:

$$\Omega = \Phi\sigma^2_G + \Pi\sigma^2_Q + I\sigma^2_E \text{ (Combined Model)}$$

where Φ is a matrix of the expected proportion of alleles that shared IBD, Π is a matrix of the proportions of alleles that actually shared IBD for a particular marker, as estimated on the basis of genotypic data, and I is an identity matrix. The parameters of these models were estimated, under the assumption that trait values followed a multivariate normal distribution, by maximizing the likelihood over all sibships, by use of a Newton-Raphson algorithm (using the "PROC MIXED" function of SAS, SAS Institute, Cary, NC). The null hypothesis of no linkage was assessed by comparing the full model to one in which σ^2_Q was constrained to equal 0, and the models were compared using a likelihood ratio test (χ^2 with one df) (16). The LOD score (LOD_{EQ}) for variance component analysis was calculated by dividing the likelihood ratio χ^2 statistic for linkage by $2*\log_e$ (10). As is conventional in linkage analysis, variance components were constrained to be ≥0. Given this constraint, the distribution of the likelihood ratio test under the null hypothesis ($\sigma^2_Q = 0$) is a mixture of distributions that is half part χ^2 with 1 df and half part point mass at 0. The one-sided P value associated with this test is well approximated by dividing that of a 1 df χ^2 by 2.

To estimate the influence of imprinting, IBD of all markers was partitioned into components reflecting marker alleles shared by siblings, derived from either mother (Π_{MO}) or father (Π_{FA}) (see below). These separate IBD terms were then included in an overall imprinting model:

$$\Omega = \Phi\sigma^2_G + \Pi_{MO}\sigma^2_{QMO} + \Pi_{FA}\sigma^2_{QFA} + I\sigma^2_E \text{ (imprinting model)}$$

allowing separate contributions of Π_{MO} and Π_{FA} to phenotypic variance. The null hypothesis of no linkage to either parent was assessed by the likelihood ratio test comparing the full model to one in which both σ^2_{QMO} and σ^2_{QFA} were constrained to equal 0. For comparison with LOD_{EQ}, the one-sided P value derived from this test (χ^2 with 2 df) is converted to an equivalent LOD score (LOD_{IMP}) (15). To test whether the model incorporating parent-of-origin effects fit the data better than the combined model, we calculated the likelihood ratio test comparing these two models and a corresponding two-tailed P value (χ^2 with 1 df; P_{DIFF}) (15).

Estimates of linkage to alleles derived from each parent were achieved by constraint of either σ^2_{QFA} or σ^2_{QMO} to 0 in the imprinting model. Thus, linkage to maternally derived alleles (reported as LOD_{MO}) was examined by comparison of the imprinting model to a model with σ^2_{QMO} constrained to 0 using the likelihood ratio test (χ^2 with 1 df). LOD scores were then calculated as above. Linkage to paternally transmitted alleles was similarly derived and reported as LOD_{FA}.

For the purposes of presentation, apart from areas of a priori interest, we report only regions where LOD scores in multipoint analysis exceeded 1.44 (pointwise $P = 0.005$ and equivalent to a Bonferroni adjusted value of $P < 0.01$) for either parent (LOD_{MO} or LOD_{FA}), as well as single-point results for markers flanking these areas of interest, calculated by the above method. Estimates of type 1 error by simulation suggest that values of P derived by the above method lie close to the nominal values (15).

Estimation of parent-specific IBD. A given pair of siblings can share 0, 1, or 2 alleles IBD ($\pi = 0$, 0.5, or 1). The number of alleles shared IBD derived from the mother can be 0 or 1 ($\pi_{MO} = 0$ or 0.5). Similarly, π_{FA} can be 0 or 0.5, and π_{MO} and π_{FA} will sum to π. When both parents are genotyped and the marker is perfectly informative, calculation of allele sharing is straightforward. However, when both parents are not genotyped or the marker is not perfectly informative, allele sharing must be estimated, given the genotypes of the sibling pair, the genotypes of the other available family members, and the population allele frequencies. For the present study, IBD estimates were obtained using the algorithm of Curtis and Sham (17), modified by us to obtain separate maternal and paternal contributions (15).

In this study, 1,916 sibling pairs for which both siblings had been genotyped were available. When possible, parental genotype was obtained either by direct genotyping (26% of fathers and 48% of mothers) or by inference from extended relatives (accomplished by the UNKNOWN program), as above. The present analyses were, thus, restricted to the 1,584 sibling pairs for whom such data were available, representing 860 individuals in 263 nuclear families. As measures of BMI were missing for some individuals, analyses of BMI were conducted across 846 individuals, in 1,526 pairs from 235 nuclear families.

Multipoint estimates of IBD were obtained by an extension of the method of Fulker et al. (18). In this method, π at any point on the chromosome is estimated as a weighted sum of π at each of the individual markers: the weights are determined by regression analysis with the constraint that the mean value of π is equal to its expected value of 0.5. For the present analysis, the weights were estimated from the total IBD distributions (π) with the constraint that the mean value of $\pi_{MO} = 0.25$ and the mean value of $\pi_{FA} = 0.25$. This ensures that $\pi_{MO} + \pi_{FA} = \pi$ for the multipoint distributions of IBD.

Affected sibling-pair analyses of diabetes. To further assess the relationship of marker alleles to diabetes, we performed an affected sibling-pair analysis by modification of the method of Elston (19). This analysis is usually conducted without consideration of parent-of-origin effects to examine the hypothesis that IBD in affected sibling pairs is >0.5 against the null hypothesis that it equals 0.5. In our analysis, we postulated that if an imprinted gene were increasing the risk of diabetes, then IBD of alleles from the parent contributing the allele usually expressed physiologically would be >0.25 (on a range of 0–0.5 as above) and IBD at the same location of alleles contributed by the parent with silenced alleles should equal 0.25. We tested this hypothesis first by examining whether π_{MO} or π_{FA} was significantly >0.25 (by unpaired t test with P values converted to LOD_{MO} and LOD_{FA}) and whether there was a

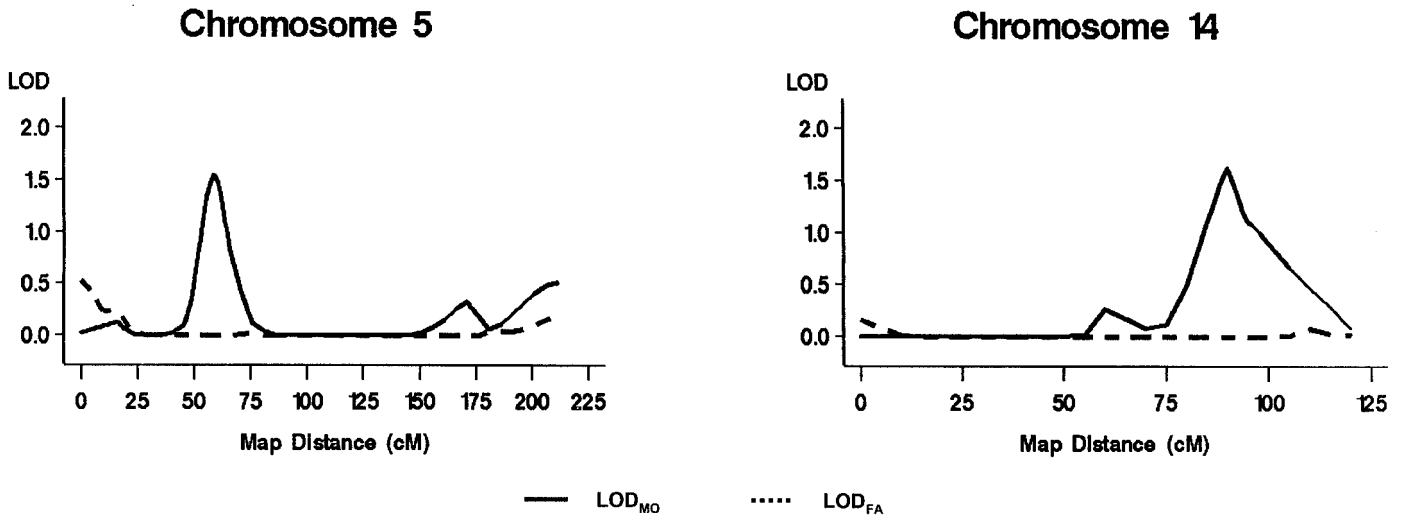

FIG. 1. Multipoint linkage to age-adjusted diabetes adjusted for age and sex by a cumulative incidence method. Linkage to maternally (LOD_{MO}) and paternally (LOD_{FA}) derived alleles.

significant difference between π_{MO} and π_{FA} (by examining whether $\pi_{MO} - \pi_{FA}$ was different from 0 by paired *t* test, expressed as P_{DIFF}). We report results for two groups of affected sibling pairs: those for whom age of onset of both siblings was <25 years (44 pairs) and those for whom age of onset was <45 years (409 pairs). The choice of the particular age limits is essentially arbitrary but does conform to ages previously selected for affected sibling-pair analysis in this population (10).

RESULTS

Multipoint linkage to age-adjusted diabetes. Two regions (Fig. 1) displayed evidence of linkage (LOD >1.44) of diabetes to alleles derived from either mother (LOD_{MO}) or father (LOD_{FA}). In both regions, stronger linkage to maternally derived alleles was found (chromosome 5 LOD_{MO} = 1.5, chromosome 14 LOD_{MO} = 1.6). For both chromosomal regions, the LOD score of the overall imprinted model (LOD_{IMP}) was only slightly above 1, and the difference between the imprinted (LOD_{IMP}) and combined (LOD_{EQ}) models was of only marginal significance (Table 1).

On a priori grounds, we were interested in the possibility of linkage to areas of the genome linked either with diabetes in the larger set of families included in the non–parent-specific genome-wide scan or with syndromic causes of obesity or diabetes that show imprinting effects. These include regions on chromosome 1 (map position 191 cM, identified as a diabetes susceptibility locus in affected sibling-pair analysis), chromosome 11 (map position 139 cM linked to both diabetes and BMI and the short arm of chromosome 11 at sites of the *INS* gene and known imprinted genes such as *IGF-2*), chromosome 6 (map position 128 cM, linked to diabetes and as a position close to the proposed locus of TNDM), and chromosome 15 (site of the Prader-Willi locus). On chromosome 11q there was a modest degree of linkage to diabetes (LOD_{EQ} = 0.9), with no evidence of imprinting effects (P_{DIFF} = 0.9). The previously noted region on chromosome 6 at 128 cM showed only very weak linkage to diabetes in the present set of families (LOD_{EQ} = 0.5), although there was a trend toward a greater contribution from paternally rather than maternally derived alleles (LOD_{IMP} = 0.8, LOD_{FA} = 0.9, LOD_{MO} = 0.1). However, the imprinting model was not significantly superior to the combined model (P_{DIFF} = 0.4). There was no evidence of linkage in other regions of interest (on chromosomes 1, 15, and 11p) with multipoint LOD_{EQ}, LOD_{FA}, and LOD_{MO} all <0.3 in these regions.

Affected sibling-pair analysis. We examined the sharing of alleles derived from father and mother in 44 pairs of offspring in whom age of onset of diabetes was <25 years and in 409 pairs with age of onset <45 years.

In sibling pairs with onset <25 years, linkage was demonstrated to either mother or father in three regions

TABLE 1
Multipoint linkage of diabetes*

Chromosome	Map distance (cM)†	Imprinting model			Combined model	P_{DIFF}	Flanking markers (map positions†)
		LOD_{MO}	LOD_{FA}	LOD_{IMP}	LOD_{EQ}		
5	59	1.5	0	1.04	0.8	0.058	D5S1470 (56.2 cM)-D5S426 (59.7 cM)
14	90	1.6	0	1.1	0.9	0.058	D14S617 (89.9 cM)-D14S744 (94.4 cM)

Multipoint linkage assessing identity by descent in sibling pairs of alleles derived either from the father (π_{FA}) or the mother (π_{MO}) against age- and sex-adjusted diabetes. Results are reported for *1*) a combined model where IBD = $\pi_{FA} + \pi_{MO}$ (LOD_{EQ}), and *2*) an imprinting model allowing separate assessment of maternal (π_{MO}) and paternal (π_{FA}) effects, in this model, the LOD score for the whole model (LOD_{IMP}) and for contributions of π_{FA} (LOD_{FA}) and π_{MO} (LOD_{MO}) are reported as well as the statistical significance of the imprinting over the combined model (P_{DIFF}). Results are reported for the combined model where either LOD_{FA} or LOD_{MO} is >1.44 ($P < 0.005$). *Diabetes is adjusted for age and sex by a cumulative incidence method. †Map positions are approximate locations for each region generated for this dataset, based on the nearby markers and available genetic maps.

TABLE 2
Analysis of pairs of affected siblings

	Map distance (cM)	π_{MO}	π_{FA}	LOD_{MO}	LOD_{FA}	P_{DIFF}	Flanking markers (map positions*)
Age of onset <25 years†							
Chromosome 1	192	0.305	0.341	0.7	2.6	0.25	D1S1589 (181.4 cM)-D1S2127 (192.4 cM)
Chromosome 6	91	0.374	0.183	3.0	0	<0.001	D6S1056 (90.9 cM)-D6S1021 (97.7 cM)
Chromosome 17	139	0.264	0.319	0	1.6	0.097	D17S784 (137.6 cM)-qter
Age of onset <45 years‡							
Chromosome 5	18	0.248	0.278	0	1.7	0.021	D5S2505 (16.7 cM)-D5S807 (23.3 cM)

*Map positions are approximate locations for each region generated for this dataset, based on the nearby markers and available genetic maps. Analysis of pairs of siblings where both siblings were affected by diabetes († under the age of 25 [$n = 44$] or ‡ under the age of 45 [$n = 409$]). Estimates of mean identity by descent of alleles derived from mother (π_{MO}) and father (π_{FA}) are given along with tests of whether π_{MO} or π_{FA} is significantly greater than the mean value expected by chance: 0.25 (expressed as LOD_{MO} and LOD_{FA}, respectively). A value for the difference between π_{MO} and π_{FA} is also given (P_{DIFF}). Results are reported where either LOD_{FA} or LOD_{MO} is >1.44 ($P < 0.005$).

(Table 2). The region on chromosome 1 (at 191 cM close to D1S2127) was reported in the previous (non–parent-of-origin–specific) analysis in this population (10). In this analysis, there was a slightly greater sharing of paternally derived alleles ($LOD_{FA} = 2.6$) than of maternally derived alleles ($LOD_{MO} = 0.7$). There was no evidence of a significant difference between π_{MO} and π_{FA} ($P_{DIFF} = 0.25$) and consequently little evidence of imprinting effects. Our strongest evidence of parent-specific linkage was on chromosome 6 with evidence of significant sharing of maternally derived alleles in sibling pairs affected before the age of 25 (Fig. 2). Finally, an excess of sharing of paternally derived alleles was observed on chromosome 17; LOD_{FA} in this instance was only 1.6, however, and the difference between alleles derived from either parent and shared between siblings was not significant.

In sibling pairs with onset at <45 years, only a single site, on chromosome 5, displayed evidence of significant linkage with an excess of sibling sharing of paternally derived alleles (Table 2; $LOD_{FA} = 1.7$). In a previous analysis using a slightly larger group including these subjects, linkage was found on chromosome 7 (at 115 cM close to D7S1799) (10). This was not reproduced in analysis of this smaller group ($LOD_{FA} = 0.1$, $LOD_{MO} = 0$).

Multipoint linkage to maximum BMI. Evidence of linkage of BMI to alleles derived from either mother (LOD_{MO}) or father (LOD_{FA}) was found in three regions (Table 3). Linkage of BMI to maternally derived alleles was found on chromosome 5 (Fig. 3) with a peak of LOD_{MO} of 1.7 at 71 cM in a region some 12 cM centromeric from the peak of linkage of maternally derived alleles to diabetes described above. Conversely, on chromosome 10 (Fig. 3), stronger linkage to paternally ($LOD_{FA} = 1.7$) as opposed to maternally ($LOD_{MO} = 0$) derived alleles was observed. In contrast to the findings for diabetes, the imprinted model fit the data significantly better than the combined model in both cases (Table 3).

Overall linkage to BMI on chromosome 11q, as previously reported in a slightly larger group that contained these subjects, was again present in this analysis, with the peak of non–parent-specific linkage at 141 cM ($LOD_{EQ} = 2.7$). There was no evidence of imprinting effects: LOD in the imprinted model was consistently lower than the combined model throughout the region (a consequence of the extra degree of freedom in the likelihood ratio test). Linkage to paternal alleles was generally higher than to maternally derived alleles; the highest value of LOD_{FA} was 1.9 at 137 cM and of LOD_{MO} was 0.6 at 150 cM. However, there was no significant difference in the effects of maternally or paternally derived loci at any point in the region. No evidence of linkage was found in other areas of a priori interest (LOD_{EQ}, LOD_{FA}, and LOD_{MO} were <0.3 in these regions; not shown).

DISCUSSION

Several authors have speculated about the potential importance of imprinting effects in type 2 diabetes and obesity (1,2). This study is the first attempt to test that hypothesis on a genome-wide basis in a large population sample. Importantly, none of our individual multipoint linkage results meets accepted criteria for statistical significance (20), despite being potentially inflated by separate consideration of LOD_{MO} and LOD_{FA} (21). The present analyses therefore do not establish that imprinted loci influence the risk of diabetes or obesity in the Pima Indian population. It remains possible that some of the regions where tentative linkage was detected reflect the actions of imprinted genes with, perhaps, relatively modest effects. None of these tentative regions lies in sites of a priori interest, although weak evidence of linkage with paternal

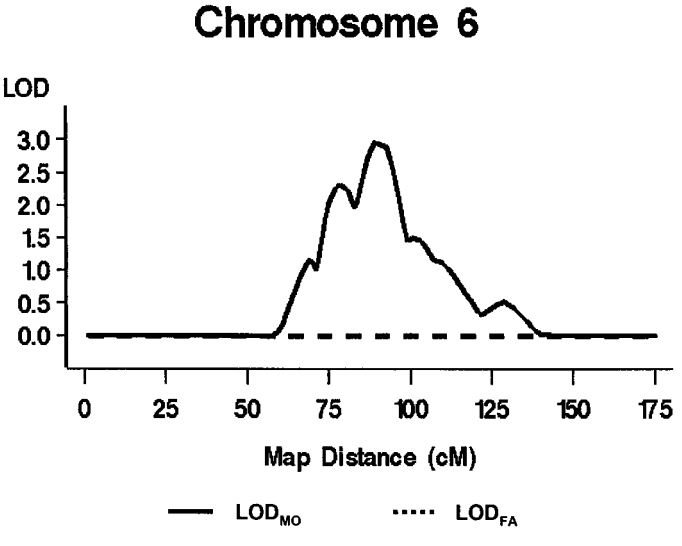

FIG. 2. Linkage to diabetes in affected sibling pairs. Pairs of siblings are included where both siblings were affected by diabetes and were younger than 25 years ($n = 44$). LOD scores act as measures of excess sharing of alleles derived from either the mother (LOD_{MO}) or the father (LOD_{FA}) in pairs of affected siblings.

TABLE 3
Multipoint linkage of BMI*

Chromosome	Map distance (cM)†	Imprinting model			Combined model	P_{DIFF}	Flanking markers (map positions†)
		LOD_{MO}	LOD_{FA}	LOD_{IMP}	LOD_{EQ}		
5	71	1.7	0	1.2	0.1	0.007	D5S2489 (67.4 cM)-D5S2500 (75.4 cM)
10	20	0	1.7	1.1	0.8	0.04	D10S1435 (6 cM)-D10S189 (19.7 cM)
11	141	0.5	1.6	2.1	2.7	0.6	D11S1998 (127.5 cM)-D11S4464 (136.6 cM)

Multipoint Linkage assessing identity by descent in sibling pairs of alleles derived from either father (π_{FA}) or mother (π_{MO}) against BMI. Results are reported for 1) a combined model where IBD = $\pi_{FA} + \pi_{MO}$ (LOD_{EQ}) and 2) an imprinting model allowing separate assessment of maternal (π_{MO}) and paternal (π_{FA}) effects, in this model, the LOD score for the whole model (LOD_{IMP}) and for contributions of π_{FA} (LOD_{FA}) and π_{MO} (LOD_{MO}) are reported as well as the statistical significance of the imprinting over the combined model (P_{DIFF}). Results are reported for the combined model where either LOD_{FA} or LOD_{MO} is >1.44 ($P < 0.005$). *Maximum BMI in subjects aged >15 years after adjustment for age and sex by linear regression. †Map positions are approximate locations for each region generated for this dataset, based on the nearby markers and available genetic maps.

alleles was observed near the putative site of TNDM on chromosome 6q. Of these a priori sites, chromosome 11p has received the most attention because of the reported association of type 2 diabetes with paternally transmitted alleles of INS VNTR III (2) and because of the presence of imprinted genes of potential importance in determination of birth weight (5,6). We found no evidence of linkage of age-adjusted diabetes score or BMI to either maternal or paternal alleles in this area. This reduces the likelihood of but cannot eliminate the possibility of an imprinted gene influencing diabetes in this region. Linkage studies of typical size (such as the present study) generally lack power to detect loci of moderate effect (accounting for <10% of phenotypic variance) and require loci of at least moderate effect (≥20% of phenotypic variance) to detect strong evidence of linkage. Simulation studies suggest that power of the present set of families to detect imprinted loci is comparable to this (15).

The areas with the most potential to form sites of imprinted genes that contribute to diabetes or obesity in this population lie on chromosomes 5, 6, and 10. Chromosome 6 was the site of the highest parent-specific LOD score with diabetes. Chromosome 5 is of interest as nearby peaks (separated by 12 cM) showed linkage to maternally derived alleles to diabetes (LOD_{MO} = 1.5) and obesity (LOD_{MO} = 1.7). Chromosome 10 is the only other site where the imprinted model was significantly superior to the combined model, in this case in linkage to BMI. What other evidence might support the presence of genes promoting diabetes or obesity and subject to imprinting in these regions? The results of previous genome-wide scans analyzed without parent-of-origin effects may contribute. If imprinted genes were present and affecting the likelihood of diabetes or obesity, then this may have been detected in an attenuated form in earlier non–parent-specific linkage analyses to either diabetes or prediabetic phenotypes. While the present methods allow one to assess the null hypothesis of no imprinting effect on the trait, it is important also to consider other lines of evidence for imprinted genes in these regions. The presence of imprinted genes may be suggested by phenotypic effects of uniparental disomy—offspring with a normal total complement of chromosomes but deriving both copies of one chromosome from a single parent (although such phenotypic effects may also arise as a result of recessive genes). Conversely, the presence of uniparental disomy in the absence of phenotypic abnormality argues against imprinted genes being present.

For chromosome 5, these other lines of evidence are not generally supportive of an imprinted locus in this region. Firstly, it should be noted that the region on chromosome 5 reported in the affected sibling-pair analysis is not supportive of the result obtained in analysis of age-adjusted diabetes: it is 40 cM distant and suggests an

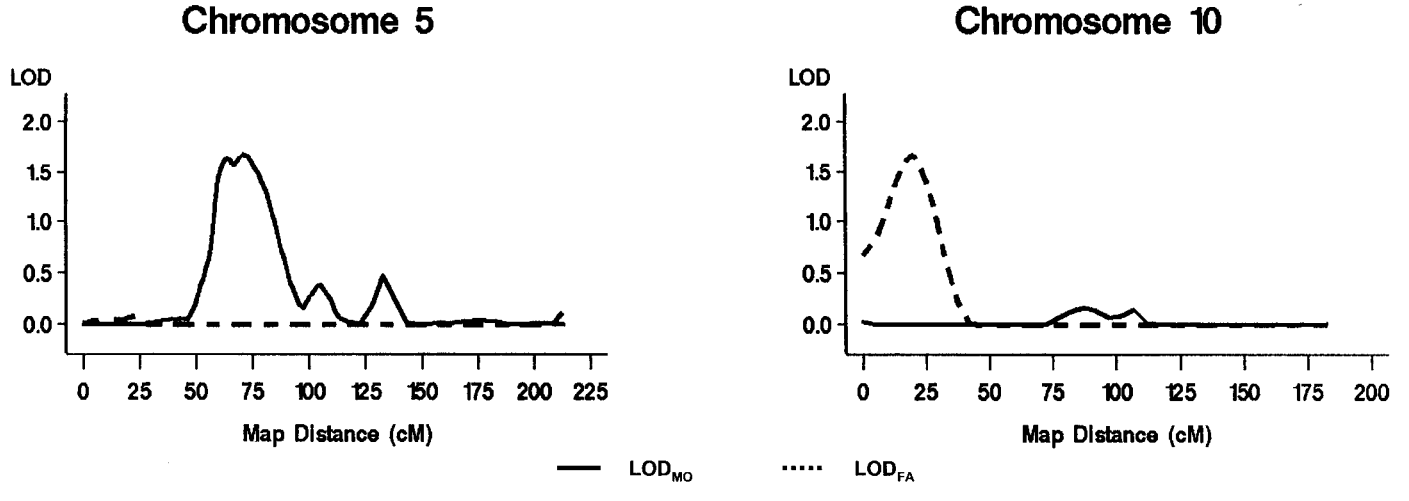

FIG. 3. Multipoint linkage to maximum BMI in subjects aged 15 years (after adjustment for age and sex by linear regression). Linkage to maternally (LOD_{MO}) and paternally (LOD_{FA}) derived alleles.

excess of sharing of alleles derived from father rather than mother. Secondly, markers on chromosome 5p did not display significant linkage to age-adjusted diabetes or BMI (10) or to prediabetic phenotypes (12) in previous, non–parent-specific genome-wide scans in the Pima population. However, linkage of obesity or diabetes has been previously noted on chromosome 5p in other populations. Linkage of obesity (LOD score = 1.57 at marker D5S647) was found in a French population sample (22), lying ~10 cM from the peak we are describing. Linkage to diabetes has been reported for chromosome 5p (LOD = 3.26, marker D5S1404) in white subjects in the GENNID study in a region ~13 cM telomeric to the BMI peak and 26 cM telomeric from the peak of linkage to diabetes we are describing (23). Finally, no known imprinted genes are reported on chromosome 5 in a recent catalogue (6). For uniparental disomy, a single case report described a case of recessive spinal muscular atrophy associated with paternal uniparental disomy of chromosome 5 (24). Importantly, apart from the recessive condition, no other phenotypic abnormalities were detailed, making significant imprinting effects unlikely (25). Although subtle phenotypes might have been masked by the neurological condition, this report does not support the presence of an important, exclusively maternally expressed gene in our region of interest on chromosome 5. Clearly, these pieces of evidence are far from precluding the existence of an imprinted gene in this area. Effects of an imprinted gene might not have been detected in a non–parent-specific linkage analysis, not all imprinted genes occur in clusters, and metabolic abnormalities may have been missed in the case of uniparental disomy.

BMI was also significantly linked to an area on chromosome 10. This region is of interest as chromosome 10p was previously reported as the putative site of a major gene affecting BMI in an affected sibling-pair analysis in a French population (22). It should be noted, however, that their region of interest, some 55 cM from the p telomere on their map (22), is ~30 cM centromeric to the region that we are describing. Chromosome 10p was not previously found to be linked to diabetes, obesity (10), or prediabetic phenotypes (12) in Pimas. Putative parent-of-origin effects have not been reported for chromosome 10 (6).

By contrast, it seems highly likely that imprinted genes are present on chromosome 6 and, furthermore, that such genes might affect glucose homeostasis. The best current evidence is for importance of paternally expressed genes. Paternal uniparental disomy has been associated with TNDM (1), and although the precise genetic cause of TNDM is not yet known, current findings suggest that it may be caused by effects of multiple copies of a paternally expressed allele of an imprinted gene. The second strongest area of linkage of age-adjusted diabetes in the previous non–parent-specific scan (10) was in the same region as the putative region of interest of TNDM on chromosome 6. In this context, our observation of weak linkage in this, one of our areas of a priori interest to paternal alleles (LOD_{FA} = 0.9), is intriguing but clearly remains inconclusive. The gene for TNDM had previously been mapped to a 3–4 cM region on chromosome 6q 24.1–24.3, between D6S1699 and D6S1010 (1). This was more recently refined to a <1 cM region at the telomeric end of this region flanked by D6S308 and D6S1010 (26). Of the markers included in our analysis, D6S1009 lies centromeric to D6S1699, whereas D6S1003 lies within the refined area of interest for TNDM, being the only one of our markers within this area. The peak of linkage (at 128 cM on the Pima map) that we observed lies between D6S1009 and D6S1003. We also observed a stronger degree of linkage of diabetes to maternally derived alleles in affected siblings under the age of 25 years to a region around 81 cM on chromosome 6. In the variance components linkage analysis of all sibling pairs, this region showed a weak degree of linkage to maternally derived alleles, falling below the cutoff (LOD >1.44) for reporting that we set ourselves. In this region, stronger linkage to maternally derived alleles was also present (LOD_{MO} = 1.2, LOD_{FA} = 0) and the imprinted model was significantly superior to the combined model in the same region (LOD_{IMP} = 1.04, LOD_{EQ} = 0, P_{DIFF} = 0.02). It is unlikely that genes involved in TNDM underpin this finding, both because the region of interest is distant and TNDM is associated with paternal rather than maternal alleles. Other imprinted genes may be acting, however; a single report records a case of agenesis of pancreatic β-cells associated with paternal uniparental isodisomy of chromosome 6 (27). The phenotype seems to be distinct from TNDM (28), and this may suggest the presence of other imprinted genes on chromosome 6 in this case involved in β-cell differentiation (28).

A number of groups have suggested linkage to areas of chromosome 20 (29,30). Previous analysis of the present data using a modification of Haseman-Elston procedure suggested parent-specific linkage on chromosome 20q, with significant linkage to paternally derived alleles (LOD_{FA} = 1.7) (31). The present variance components analysis also indicated a degree of linkage to paternally derived alleles (LOD_{FA} = 1.3), however, without a significant difference from maternally derived alleles (P_{DIFF} = 0.12).

For all of the regions of interest that we have reported, it is also important to consider whether the evidence for imprinting effects that we report may arise as an artifact. The genetic maps used for multipoint linkage analysis in this and other studies use sex-averaged genetic distances. Recombination rates differ between the sexes, however, and it has been suggested that this may lead to artifactual evidence of imprinting (32). For example, in areas of the genome where recombination rates are higher in genetic material transmitted from mothers than from fathers (with corresponding increase in female map distances), a spurious linkage to paternally derived alleles may arise. This does not seem to be the explanation for our results. In almost all of the regions where evidence for imprinting was detected (on chromosomes 5, 9, and 10 in all siblings and chromosomes 5, 6, and 17 in affected sibling-pair analysis), recombination rates for the parent showing linkage were higher than those of the other parent (data not shown), indicating that effects of sex-specific recombination do not explain the results. The only exception to this is the region on chromosome 6 close to the TNDM locus discussed above, where recombination rates were lower for males than for females (map distances between markers D6S1009 and D6S1003 17.1 cM for females and 6.8 for males). It remains possible that this peak may be

inflated artifactually as a result of lower recombination rates in paternally transmitted genetic material. Another potential source of artifact in variance components analysis derives from deviations of phenotypic traits from multivariate normality with potential inflation of the type 1 error rate (33). Of the traits used in this analysis, BMI (age- and sex-adjusted) is close to a normal distribution (Shapiro-Wilk statistic 0.99), whereas the diabetes score is, by design, bimodal. Transformation to normality forms a possible approach to this but is not considered ideal because of the potential to increase in type 2 error (33). For this reason, we used untransformed phenotypes for detailed reporting of results. Transformation of phenotypes to normality resulted in similar results (data not shown), suggesting that these peaks do not rise simply as a result of artifact from this source.

In conclusion, we examined the hypothesis that imprinted genes might be involved in predisposition to type 2 diabetes and obesity in a group of Pima Indians. Tentative evidence for imprinting effects was detected on chromosomes 5, 6, and 10. In that light, the two regions on chromosome 6 may be the most interesting, as independent evidence supports the possibility of imprinted genes affecting glucose tolerance on this chromosome. In addition, we detailed important negative evidence. We found no evidence of imprinted effects at other sites of potential action of imprinted genes in type 2 diabetes, such as chromosome 11p, or for loci detected in the previously reported non–parent-specific genome-wide scan.

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Electronic database information: Accession numbers and the URL for data in this article are found at Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/htbin-post/Omim/ (for Prader-Willi [MIM 176270] and Transient Neonatal Diabetes Mellitus [MIM 601410]).

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