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Genome-wide linkage analysis assessing parent-of-origin effects in the inheritance of birth weight

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Abstract Family studies suggest that genetic variation may influence birth weight. We have assessed linkage of birth weight in a genome-wide scan in 269 Pima Indian siblings (334 sibling pairs, 92 families). As imprinting (expression of only a single copy of a gene depending on parent-of-origin), is commonly found in genes that affect fetal growth, we used a recently described modification of standard multipoint variance-component methods of linkage analysis of quantitative traits. This technique allows for comparison of linkage models that incorporate imprinting effects (in which the strength of linkage is expressed as LOD_{IMP}) and models where parent-of-origin effects are not included (LOD_{EQ}). Where significant evidence of linkage was present, separate contributions of alleles derived from father (LOD_{FA}) or mother (LOD_{MO}) to the imprinting model were estimated. Significant evidence of linkage was found on chromosome 11 (at map position 88 cM, LOD_{IMP}=3.4) with evidence for imprinting (imprinting model superior, P<0.001). In this region, birth weight was linked predominantly to paternally derived alleles (LOD_{FA}=4.1, LOD_{MO}=0.0). An imprinted gene on chromosome 11 may influence birth weight in the Pima population. This chromosome contains one of the two major known clusters of imprinted genes in the human genome, lending biological plausibility to our findings.

Introduction

Birth weight is affected by both genetic and environmental factors. Environmental influences leading to lower birth weight include maternal smoking, hypoxia, extreme maternal under-nutrition, and the presence of multiple pregnancies (Kline et al. 1989). The influence of genes on birth weight is reflected in lower birth weight in relation to female sex and chromosomal abnormalities (Kline et al. 1989). Furthermore, disruption of single genes, particularly those affecting the actions of insulin and insulin-like growth factor 2 (IGF2) in animal models also lead to reduction of birth weight (Efstratiadis 1998). These studies also highlight an important role of imprinted genes, viz., genes in which only a single copy is expressed depending on parent of origin, in influencing fetal growth and development (Efstratiadis 1998; Reik and Walter 2001).

The relative importance of environmental and genetic influences on variation in birth weight in populations is not clear. Heritability of birth weight has been estimated in family studies at between 30% and 70% (Baird et al. 2001; Clausson et al. 2000; Magnus 1984a, 1984b; Nance et al. 1983). Whereas this is usually interpreted as supporting genetic effects, effects of shared environment cannot be excluded (Magnus et al. 1984).

Birth weight has long been recognized as a clinical indicator of perinatal survival and infant mortality (Leon 1991). More recently, interest in the factors that might influence birth weight has increased because of associations of birth weight with common chronic diseases (Barker 1992). In particular, lower birth weight has been associated with cardiovascular disease, hypertension, type 2 diabetes, and dyslipidemia in adult life (Barker 1992). It has been hypothesized that the association of birth weight and later disease might be explained by pleiotropic effects of genes acting on both birth weight and on risk of later disease (Hattersley and Tooke 1999; Stern et al. 2000). In keeping with this model, mutations of the glucokinase gene responsible for the early onset of diabetes in type 2 maturity onset diabetes of the young (MODY 2) are also associated with lower birth weight (Hattersley et al. 1998).

The Pima Indians of Arizona suffer from an extremely high prevalence of type 2 diabetes and obesity (Knowler et al. 1978, 1991), conditions that appear to have a major

Electronic database information:
The Imprinted Gene Catalogue by I. M. Morison is found at http://cancer.otago.ac.nz/IGC/web/home.html

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genetic component. In the Pima population, there is a U-shaped relationship of birth weight with later risk of type 2 diabetes, with newborns of higher and lower birth weights being at higher risk than those at the center of the distribution (McCance et al. 1994). Lower birth weight is also associated with an excess of paternal diabetes, suggesting the possibility that imprinted genes influence both diabetes risk and birth weight (Lindsay et al. 2000a).

Given the possibility that genes influencing birth weight might also contribute to type 2 diabetes, we have examined which chromosomal regions might influence birth weight in this population by using genome-wide linkage analysis. Recently developed modifications of existing linkage techniques (Hanson et al. 2001) have also allowed examination of the hypothesis that parent-of-origin effects influence linkage of birth weight to genetic markers in this population.

Subjects and methods

Subjects and phenotypes

The subjects of this report are participants in the National Institutes of Health survey of health in the Gila River Indian Community. Members of the community over the age of 5 years are invited to participate in research examinations, including a biennial oral glucose tolerance test (OGTT) after a 75-g glucose load (Knowler et al. 1978). In keeping with previous analyses in this population, diabetes is diagnosed if fasting blood glucose is ≥140 mg/dl glucose, 2 h after the 75-g glucose load, is ≥200 mg/dl, or if diabetes has been diagnosed in a clinical setting (World Health Organisation 1985). Birth weights and gestational age were ascertained by review of medical records. Subjects were included if they were singleton births, both birth weight and gestational age were available, and gestational age was ≥33 weeks. As the presence of maternal diabetes influences birth weight due to environmental effects of maternal hyperglycaemia exerted on the fetus *in utero* (Pettitt et al. 1980), and a small secular increase in birth weight has been described in this population (Lindsay et al. 2000b), adjustments for maternal diabetes and year of birth were made. Children were considered as being: (1) offspring of diabetic mothers (ODM) if their mother had been diagnosed as having type 2 diabetes by World Health Organisation criteria (World Health Organisation 1985), either before or during the index pregnancy, (2) offspring of non-diabetic mothers (ONDM) if their mother was known to have a non-diabetic OGTT after the index pregnancy, (3) possible offspring of diabetic mothers (POSS) where maternal diabetes had been diagnosed after the birth of the child without an intervening non-diabetic OGTT after the birth of the child, rendering the exact time of onset of diabetes uncertain, (4) offspring of unknown status (UNK) where maternal diabetes status had not been assessed. Prior to linkage analysis, birth weights were adjusted for gender, gestational age (as linear and quadratic terms), birth year, and maternal diabetes status (with indicator variables for the different categories) using data from 4076 births in the Pima population (246 DIAB, 2943 NON, 304 POSS, 583 UNK) by means of linear regression.

Informed consent was obtained from all subjects, and ethical approval was received from both the National Institutes of Health and the Gila River Indian Community.

Genotyping

A sample of 1338 individuals (in 332 nuclear families) who had participated in the longitudinal study were selected for genomic scans for loci linked to type 2 diabetes and obesity (Hanson et al. 1998). Criteria for inclusion were availability of DNA and membership of a nuclear family informative for diabetes or its metabolic correlates. The subjects of this report are a subset of this larger series; the birth weight and age of gestation at birth were also available for at least two siblings from each family. A total of 269 individuals (334 sibling pairs) in 92 families met these restrictions, with a median of two siblings per family (range 2–7). Twelve were offspring of mothers with type 2 diabetes during pregnancy (mean±SD birth weight: 4184±667 g), 200 of non-diabetic mothers (birth weight: 3601±431 g) and 54 possible offspring of diabetic mothers (birth weight: 3763±491 g). In four cases, the diabetic status of mother was undefined (birth weight: 3627±431 g).

Genotypic data used were identical to those in a previous report (Hanson et al. 1998). In brief, 503 autosomal microsatellite markers were typed in the laboratory of J. Weber, at the Marshfield Medical Research Foundation (Dubovsky et al. 1995). 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 of less than 90%. All markers were inspected for Mendelian errors, and genetic distances between markers were determined from the Pima data as previously described (Hanson et al. 1998).

Linkage analysis

Linkage analysis was conducted by a modification of the variance-components method of Amos (1994). A series of "mixed" models was fitted to allow the assessment of imprinted and non-imprinted effects. Conventionally, these models estimate the trait mean (μ) and three components of variance without imprinting effects. Variance is 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). Under the assumption of no recombination between the trait and marker loci, the phenotypic variance-covariance matrix (Ω) for individuals in a pedigree is:

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

where Φ is a matrix of the expected proportion of alleles shared identical by descent (IBD), Π is a matrix of the proportions of alleles 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, on the assumption that the distribution of the trait was multivariate normal, by maximizing the likelihood over all sibships, with a Newton-Raphson algorithm ("PROC MIXED" function of SAS, SAS Institute, Cary, N.C., USA). 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 by using a likelihood ratio test (which under the null hypothesis is distributed as a 1/2: 1/2 mix of χ^2 with 1 df and a point mass at 0; Hopper and Mathews 1982). The LOD score was calculated by dividing the likelihood ratio test for linkage by $2*\log_e(10)$. As this conventional analysis assumes equal maternal and paternal contributions at the quantitative trait locus, this LOD score will be termed "LOD_{EQ}".

To estimate the influence of imprinting, IBD of all markers was partitioned into components reflecting alleles shared by siblings and derived from either the mother (π_{MO}) or father (π_{FA}) (*v.i.*). These separate IBD terms were then entered into an overall imprinting model:

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

This model estimates four variance components and allows for separate maternal and paternal contributions at the quantitative trait locus (σ^2_{QMO}, σ^2_{QFA}). Statistical significance in this model was measured (1) by using a null hypothesis of no linkage with either paternal or maternal chromosomes (σ^2_{QMO} and σ^2_{QFA} constrained to 0), compared by the likelihood ratio test and reported as a LOD score and (2) against the non-imprinted ("combined") model by likelihood ratio test (χ^2, 1 df) and reported as a *P* value (P_{DIFF}). The latter test assesses whether a parent-of-origin model fits the

data better than a model with no parent-of-origin effect. Under the null hypothesis, the former test has a distribution that is 1/4 part χ^2 with 2 d.f., 1/2 part χ^2 with 1 d.f., and 1/4 part point mass at 0 (Hopper and Mathews 1982). Our simulation studies suggest that the *P*-value associated with this test is well-approximated by dividing that for a corresponding χ^2 with 2 d.f. by 2 in a fashion analogous to the approach that is typically used to evaluate a single component (Hanson et al. 2001). For presentational purposes and for comparison with LOD_{EQ}, these *P*-values were converted to equivalent LOD scores (based on the corresponding χ^2 test with 1 d.f. (Hanson et al. 2001)).

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 with a model with σ^2_{MO} being constrained to 0 by using the likelihood ratio test (χ^2 with 1 df). LOD scores were then calculated as for LOD_{EQ}. Linkage to paternally transmitted alleles were similarly derived and reported as LOD_{FA}.

For the purposes of presentation, apart from areas of *a priori* interest, we have reported in detail only regions where LOD scores (either LOD_{EQ} or LOD_{IMP}) in multipoint analysis exceeded 2.1 (pointwise $P<0.001$), and single point results for markers in these regions of interest, calculated by the above method.

Estimation of parent-specific IBD

Estimates of the separate contribution of maternally (π_{MO}) and paternally (π_{FA}) derived alleles to IBD were made by modification (Hanson et al. 2001) of the method of IBD estimation developed by Curtis and Sham (Curtis and Sham 1994).

For the present study, estimates were made for sibling pairs for which both siblings had been genotyped. To identify separate parental contributions, genotypic data must be available for at least one parent either by directly genotyping the parents (26% of fathers and 48% of mothers) or by inference from extended relatives (accomplished by the UNKNOWN program). Where genotyping information is available for both parents and is fully informative, π_{MO} and π_{FA} will equal either 0 or 0.5, if expressed as a proportion of the total number of alleles. In all cases, the sum of π_{MO} and π_{FA} will equal π Where genotyping information is either not available for one parent or not fully informative, then estimates of π_{MO} and π_{FA} are calculated based on allele frequencies in the entire population. A total of 269 siblings was included in the analysis (334 sibling pairs) in 92 families, with a median of two (range 2–7) siblings per family. Of 184 parents, 135 (73%) had been genotyped directly. Parental genotype information was unavailable for both parents (directly or by inference) in three families (7 sibs, 5 sibling pairs), who were included because of their potential contribution to LOD_{EQ}.

Multipoint estimates of IBD were obtained by an extension of the method of Fulker et al. (1995). 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 following constraints: 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.

Results

Multipoint and single point linkage to birthweight

In a genome-wide linkage analysis of birth weight that used both the imprinting and non-imprinting model, significant evidence of linkage (LOD_{IMP} or $LOD_{EQ}\geq 3.3$) was found only on chromosome 11 (Fig. 1). This was by far the largest LOD score: only two further areas had LOD scores greater than 1, but in both cases only marginally so (Fig. 1: chromosome 3 peak $LOD_{EQ}=1.2$; chromosome 21 peak $LOD_{EQ}=1.1$). On chromosome 11, the peak of linkage for

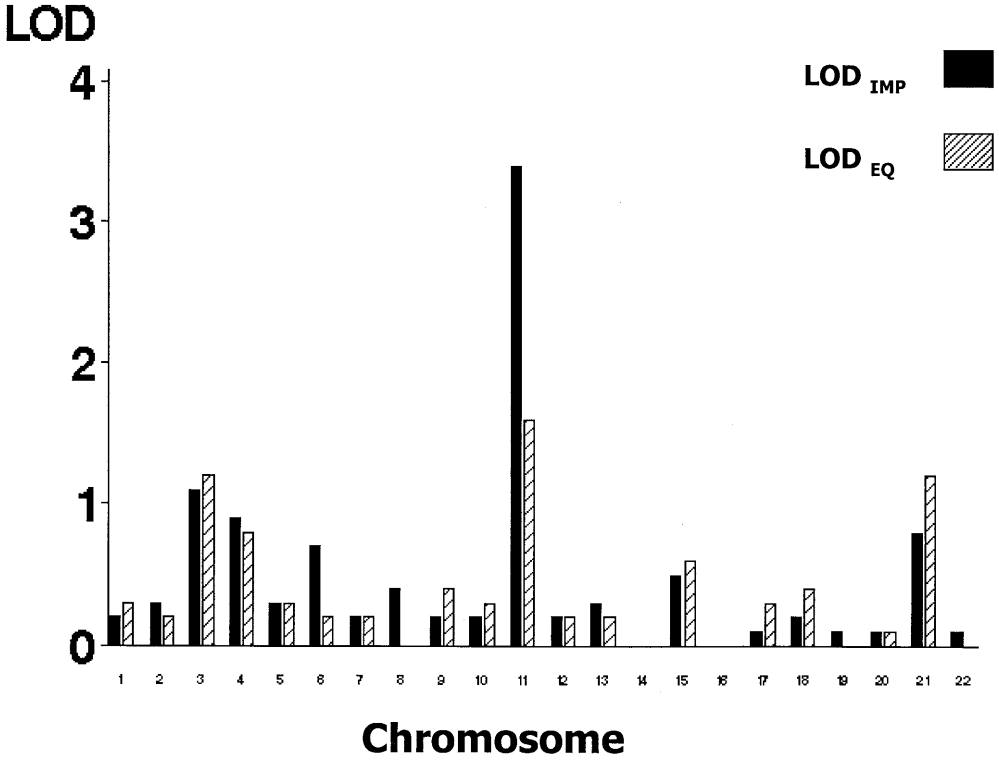

Fig. 1 Maximum multipoint linkage of birth weight (adjusted for gestational age and sex and maternal diabetes status in pregnancy). Peak of linkage for models allowing for (*solid bars* LOD_{IMP}) or not allowing for (*hatched bars* LOD_{EQ}) for imprinting effects is shown for each chromosome

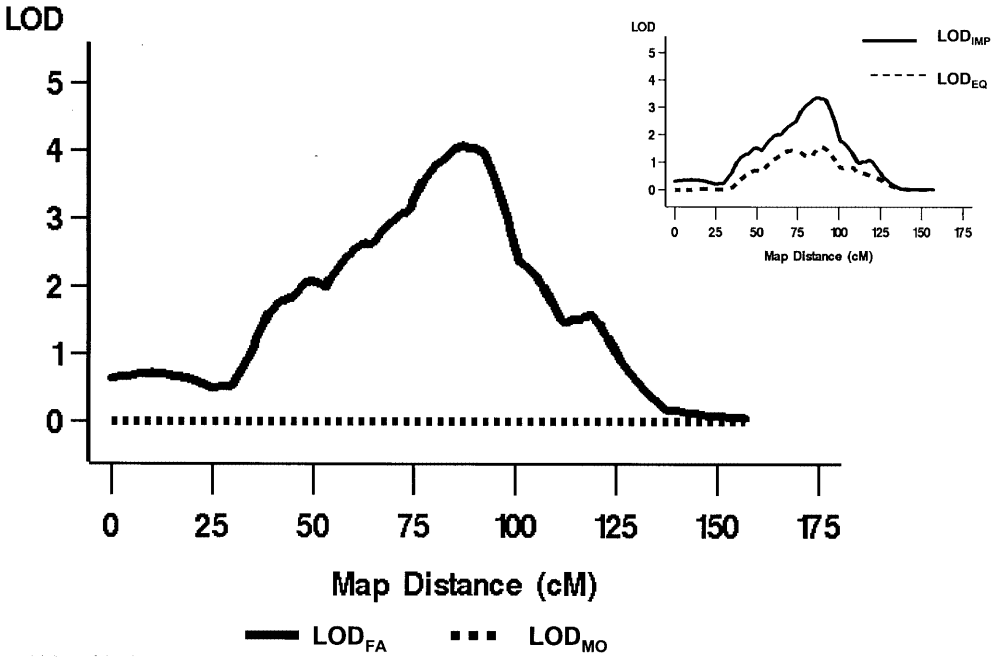

Fig. 2 Multipoint linkage to birth weight (adjusted for gestational age and sex and maternal diabetes status in pregnancy). Linkage to maternally (LOD_{MO}) and paternally (LOD_{FA}) derived alleles for chromosome 11 is shown in the main figure. *Inset* Comparison of models allowing (LOD_{IMP}) or not allowing (LOD_{EQ}) for imprinting effects

Table 1 Single point linkage of birth weight[a] to markers on chromosome 11. Single point linkage to consecutive markers between (sex averaged) map positions 50 and 110 cM. Results are reported for a combined model where IBD=$\pi_{FA}+\pi_{MO}$ (LOD_{EQ}) and an imprinting model allowing separate assessment of maternal and paternal effects where π_{FA} and π_{MO} are used as independent predictor variables. In the latter 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 is the statistical significance of the imprinting over the combined model (P_{DIFF})

Marker	Sex averaged map distance (cM)[b]	Imprinting model			Combined model		Recombination distance from preceding marker: female[b]	Recombination distance from preceding marker: male[b]
		LOD_{MO}	LOD_{FA}	LOD_{IMP}	LOD_{EQ}	P_{DIFF}		
D11S1392	52.9	0	1.0	0.6	0.3	0.06	–	–
D11S1985	63.3	0	2.7	2.1	1.1	0.007	17.7	4.1
D11S2016	64.3	0	1.0	0.6	0.6	0.2	2.1	0.2
D11S2365	65.5	0	2.1	1.6	1.2	0.04	1.5	0.7
D11S987	73.8	0	1.3	0.8	0.5	0.05	14.3	3.1
D11S1975	79	0	2.7	0.8	0.3	0.04	7.8	2.9
D11S2371	82.6	0	3.5	2.1	0.6	0.002	5.7	1.9
D11S2002	89.1	0	3.2	2.8	1.9	0.007	7.1	5.8
D11S1396	92.2	0	1.0	2.6	1.2	0.002	2.7	3.0
GATA35	101	0	1.5	0.6	0.2	0.05	10.3	7.5
D11S2000	108.7	0	1.5	1.0	1.5	0.09	12.4	3.3

[a]Birth weight adjusted for gender and gestational age by linear regression

[b]Map positions are approximate locations for each region generated for this dataset, based upon the nearby markers and available genetic maps

the imprinting model lay at 88 cM (Fig. 2: peak LOD_{IMP}= 3.4). By contrast, whereas the peak of linkage for the non-imprinted model lay in the same region, evidence for linkage under this model was much diminished (LOD_{EQ}=1.5). Comparison of the models (LOD_{IMP} vs LOD_{EQ}) suggested significant evidence of an imprinting effect (P_{DIFF}<0.001) and linkage to paternally (LOD_{FA}=4.1) but not maternally (LOD_{MO}=0) derived alleles (Fig. 2). Single point analysis revealed evidence of linkage by using the imprinting model ($LOD_{IMP}\geq$1.44, nominal *P*-value <0.005) in five of the eleven markers lying between 50 cM and 110 cM on chromosome 11 (Table 1). In all cases, this was in conjunction with evidence of linkage to paternally derived alleles, whereas by contrast, no evidence of linkage to maternally derived alleles was observed (Table 1).

To assess whether adjustment of birth weight for maternal diabetes status had influenced these results, analysis of birth weight unadjusted for maternal diabetes (but with adjustment for birth year, gestational age, and gender) was also carried out. This revealed similar results: the peak of linkage on chromosome 11 was again at 88 cM (LOD_{IMP}= 3.1, LOD_{EQ}=1.3: P_{DIFF}<0.001). To assess whether subjects contributing to the linkage peak on chromosome 11 were chiefly from one extreme of the birth weight distrib-

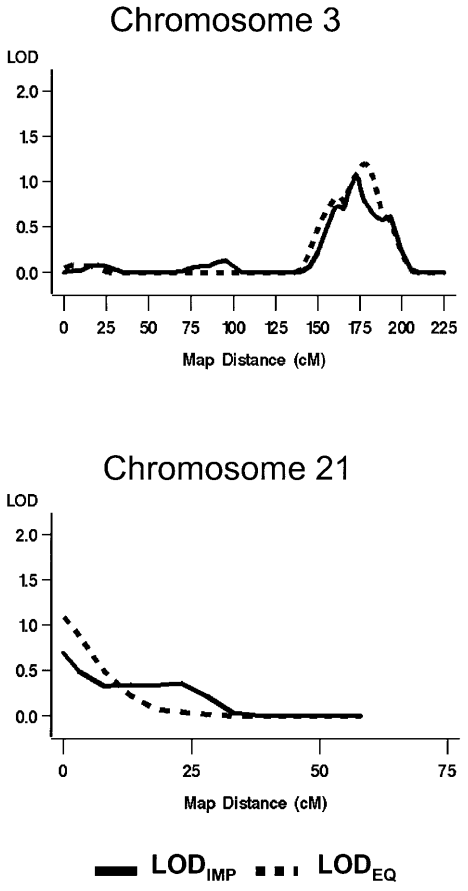

Fig. 3 Multipoint linkage to birth weight (adjusted for gestational age and sex and maternal diabetes status in pregnancy). Linkage to models either allowing for (LOD_{IMP}) or not allowing for (LOD_{EQ}) imprinting effects for chromosomes 3 and 21. Note that the scale of the *y axis* differs from those in Figs. 1, 2

ution, we restricted linkage analysis to subjects in either the upper (top three quartiles) or lower (bottom three quartiles) parts of that distribution. Linkage was considerably attenuated but remained in both cases (top three quartiles LOD_{FA} 1.5 at 88 cM; bottom three quartiles LOD_{FA} 1.9 at 88 cM), suggesting that subjects contributing to linkage were not confined to one part of the birth weight distribution.

Chromosomes 3 and 21 also displayed some evidence of linkage, albeit much weaker than that on chromosome 11 (Fig. 1). On chromosome 3, the peak of linkage lay at 176 cM with no evidence of an imprinting effect (Fig. 3: LOD_{EQ}=1.2, LOD_{IMP}=0.9, P_{DIFF}=0.2). The peak of linkage on chromosome 21 lay at the p terminus, again with no evidence of an imprinting effect (Fig. 3: LOD_{EQ}=1.1, LOD_{IMP}=0.7, P_{DIFF}=0.6).

Discussion

Birth weight shows significant linkage to chromosome 11 with evidence for an effect of an imprinted, paternally expressed gene. Chromosome 11 includes one of the two principle known clusters of imprinted genes in humans (Morison and Reeve 1998; the other being on chromosome 15), making these results of particular interest and a biological plausibility.

To our knowledge, no previous analyses have examined linkage of birth weight in a genome-wide analysis, with or without incorporation of parent-of-origin effects. A wealth of evidence points toward the importance of genetic variation on chromosome 11p in the control of human growth, and in particular, toward the action of imprinted genes. In animal models, imprinted genes including those for IGF2 (*IGF2*), the IGF2 receptor (*IGF2R*), and the insulin gene (*INS*), play a critical role in the control of growth (Efstratiadis 1998). In humans, over 30 genes are known to be imprinted, of which more than ten are found in the cluster on chromosome 11p15.5, including *IGF2* and *INS* (http://cancer.otago.ac.nz/IGC/web/home.html). *IGF2* is solely paternally expressed in humans (Giannoukakis et al. 1993). By contrast, *INS* appears to be imprinted only in certain tissues in humans (notably the fetal yolk sac) but is also hypothesized to be of importance in fetal growth (Moore et al. 2001). Influences of *IGF2* and *INS* have also been shown by examination of allelic variation at the INS variable number tandem repeat (*VNTR*) on chromosome 11p. Further, the *INS VNTR* has been associated with both birth weight (Dunger et al. 1998; Ong et al. 1999) and type 2 diabetes (Ong et al. 1999). Importantly, however, the peak of linkage that we have detected lies 88 cM from the chromosome 11p telomere, between markers D11S2371 and D11S2002, and over 80 cM distant from the known imprinted genes on chromosome 11p15.5, rendering these unlikely candidates.

Clearly, part of our purpose in examining birth weight in this population is to consider whether genetic factors might underpin the relationship of birth weight and diabetes. Not only are the individuals in the present study from a population at high risk of diabetes, but they are also subjects of a previous genome-wide screen for diabetes, often being members of families affected by type 2 diabetes. It might be expected that polymorphism influencing diabetes (and potentially birth weight) might be increased in this selection of the population. Interestingly, previous analyses of these subjects have discerned evidence of linkage of diabetes to chromosome 11 (Hanson et al. 1998); however, the peak of linkage (at 139 cM) in that study is 51 cM distant to the peak that we have demonstrated as being associated with birth weight and is unlikely to represent the same locus. We have examined whether type 2 diabetes is related to imprinting effects in a larger group of subjects from this population, including some individuals in this report (Lindsay et al. 2001). Importantly, there is little evidence of parent-specific linkage of diabetes to chromosome 11 (Lindsay et al. 2001). Thus, whereas an imprinted gene or genes on chromosome 11 may influence birth weight, there is little evidence to support a pleiotropic effect of this gene on type 2 diabetes.

A number of non-imprinted genes have been examined for relationships with birth weight in human populations, most notably the diabetes susceptibility genes. Mutations

in the glucokinase gene (which cause maturity onset type 2 diabetes of the young) are associated with lower birth weight (Hattersley et al. 1998). Although this supports the hypothesis that the connection between low birth weight and later type 2 diabetes might be underpinned by genetic factors, the rarity of these mutations in the general population makes it unlikely that they underpin a substantial proportion of variation of birth weight. Type 2 diabetes is not linked to the glucokinase gene on chromosome 7 in the Pima population (Janssen et al. 1994), and no mutations of glucokinase known to be associated with MODY-2 have been reported (Baier et al. 2000). We have found no evidence of significant linkage of birth weight on chromosome 7 in the current report. Other non-imprinted candidate genes for type 2 diabetes have been examined in other populations and found not to be associated with birth weight, including the genes for insulin receptor substrate 1, hepatocyte nuclear factor-1alpha (HNF-1alpha), HNF-4alpha, and HNF-6 (Rasmussen et al. 2000).

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 utilize 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 (Paterson et al. 1999). 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. Importantly, calculated recombination rates for females are greater than for males in this data set, as is reported in other populations (Paterson et al. 1999). However this difference is modest in the region of highest linkage that we have observed (D11S2371 to D11S2002 to D11S1396: a female genetic distance of 9.8 cM vs a male genetic distanc of 8.8 cM). Whereas the ratio of map length in females vs males is similar for other chromosomes in this data set, there nevertheless remains a theoretical possibility that the peak of linkage that we have observed has been inflated by this influence. Against this, the absence of linkage to maternally derived alleles in either multipoint or singlepoint analysis in this region appear to render this interpretation unlikely. The effect of sex-averaging on the ability to localize areas of linkage (e.g., the 1-LOD confidence interval) is not known.

Other sources of artifact may involve sample size and the distribution of the quantitative trait. The variance components method assumes multivariate normality, and deviations from this potentially lead to inflation of the type 1 error rate (Allison et al. 1999). Adjusted birth weight, however, has a distribution close to normal, and analysis of an inverse Gaussian transformation of the trait to normalize the distribution completely does not alter the results substantially (data not shown). The present sample size is relatively small (334 sibling pairs), although estimates of the type 1 error and threshold of significance for linkage analysis are reported to be robust when the total number of sibling pairs exceeds 50 (Lander and Lincoln 1988). Finally, birth weight may also be influenced by maternal genes altering the fetal environment; for example, in this population, genes affecting maternal glycemia might in turn be expected to affect birth weight. This analysis has been designed only to assess the impact of variation of fetal genes on birth weight, and we would not expect effects of maternal genes to produce spurious evidence of linkage, since variance from maternal genetic influences are partitioned into the polygenic component of the model. In keeping with this, although we have adjusted birth weight for the presence of maternal diabetes, such influences of maternal glycemia are likely to be shared by siblings, and this overall adjustment appears to have had little overall influence on the peak of linkage. Further, simulation studies suggest that even marked maternal influences also do not appear to inflate the type 1 error rate of linkage to fetal markers (Hanson et al. 2001).

In conclusion, we present evidence that paternally expressed genes on chromosome 11 influence birth weight in the Pima population.

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