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Possible Genomic Imprinting of Three Human Obesity–Related Genetic Loci

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To detect potentially imprinted, obesity-related genetic loci, we performed genomewide parent-of-origin linkage analyses under an allele-sharing model for discrete traits and under a family regression model for obesity-related quantitative traits, using a European American sample of 1,297 individuals from 260 families, with 391 microsatellite markers. We also used two smaller, independent samples for replication (a sample of 370 German individuals from 89 families and a sample of 277 African American individuals from 52 families). For discrete-trait analysis, we found evidence for a maternal effect in chromosome region 10p12 across the three samples, with LOD scores of 5.69 (single-point) and 4.52 (multipoint) for the pooled sample. For quantitative-trait analysis, we found the strongest evidence for a maternal effect (single-point LOD of 2.85; multipoint LOD of 4.01 for body mass index [BMI] and 3.69 for waist circumference) in region 12q24 and for a paternal effect (single-point LOD of 4.79; multipoint LOD of 3.72 for BMI) in region 13q32, in the European American sample. The results suggest that parent-of-origin effects, perhaps including genomic imprinting, may play a role in human obesity.

Introduction

Obesity (MIM 601665) is a common disorder and a major risk factor for type 2 diabetes mellitus, hypertension, and cardiovascular diseases (Carroll 1998; Kopelman 2000; Price 2001; Shmulewitz et al. 2001; Wolk et al. 2001). Family, twin, and adoption studies suggest that substantial family variance is genetic in origin (Stunkard et al. 1986, 1990; Sorensen et al. 1989; Grilo and Pogue-Geile 1991; Price and Gottesman 1991; Maes et al. 1997). However, major-gene effects are rare (Snyder et al. 2004). The pattern of inheritance of obesity and the rarity of mutations in known major genes suggest a complex mode of inheritance involving multiple genes.

To date, a number of linkage studies, including ~30 human-genome scans, have been reported for obesity-related phenotypes (Snyder et al. 2004), but parent-of-origin effects have been investigated only recently, with inconsistent results (Lindsay et al. 2001; Gorlova et al. 2003). To detect potentially imprinted genetic loci that influence obesity, we performed genomewide parent-of-origin linkage analysis under an allele-sharing model for discrete traits and under a family regression model for obesity-related quantitative traits, using a sample of European Americans segregating extreme obesity and normal weight. We also used two smaller, independent samples for replication of results from the genome scan.

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Subjects and Methods

Study Samples

This study included 1,297 individuals from 260 European American (EA) families and 277 individuals from 52 African American (AA) families recruited in a genetic study of obesity at the University of Pennsylvania and 370 individuals from 89 German (MB) families recruited in a genetic study of childhood and adolescent obesity at the University of Marburg. The recruitment procedures have been described elsewhere (Price et al. 1998; Lee et al. 1999; Hinney et al. 2000). In brief, for the EA and AA samples, all family probands (BMI $\geq$ 40 [BMIs measured in kg/m^2]) had at least one obese sibling (BMI $\geq$ 30) and at least one parent and one sibling with normal weight (BMI $<$ 27). All subjects in the EA and AA samples gave informed consent, and the protocol was approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania. For the MB sample, all families had at least one sibling with age- and sex-specific BMI percentile $\geq$95th, at least one sibling with age- and sex-specific BMI percentile $\geq$90th,

Table 1

Number of Sib Pairs, by Sample and Phenotype

Sample and Phenotype	No. of Sib Pairs
EA:	
BMI	1,280
%fat	940
Waist	1,103
BMI ⩾ 27	658
BMI ⩾ 30	472
AA:	
BMI	323
%fat	284
Waist	291
BMI ⩾ 27	181
BMI ⩾ 30	138
MB:	
BMI ⩾90th percentile	116

and DNA available from both biological parents. Only obese siblings were included in the MB sample. We performed a genomewide scan for parent-of-origin effects in the large sample of EA families. Because of the small size of the AA sample and the unavailability of quantitative-trait measurements for the MB sample, they were used only for replication of the results for the discrete variables.

Phenotypes

For the EA and AA samples, we used two overlapping categories of obesity status based on measured BMI (overweight BMI ⩾ 27; obese BMI ⩾ 30) and three quantitative traits (BMI, percentage of body fat [%fat], and waist circumference, after the linear effects of age within sex, race, and generation were controlled). For the MB sample, we defined "affected" as age- and sex-specific BMI percentile ⩾90th. Since the MB sample included obese siblings only, we did not perform analyses of quantitative traits for that sample.

Genotyping

A set of 382 polymorphic microsatellite markers (set 11) was genotyped for the EA and AA samples by the Marshfield Center for Medical Genetics, whereas genotyping of a set of polymorphic microsatellite markers for the MB sample was performed using a fluorescence-based semiautomated technique on automated DNA-sequencing machines. Genetic map distances were taken from the Marshfield Genetic Map Database. To fill the gaps in the regions in which we found linkage in previous studies (Lee et al. 1999; Price et al. 2001; Li et al. 2003, 2004), nine microsatellite markers (*D7S692, D10S197, D10S193, D10S1781, IGF1, D12S1339, D12S349, D20S178,* and *D20S176*) were manually genotyped for the EA and AA samples. All genotypes were checked for Mendelian inheritance by use of the program MERLIN (version 0.9), and all errors were either corrected or recoded as unknown.

Parent-Specific Linkage Analyses of Discrete Traits

We used the program ALLEGRO to assess parent-of-origin effects by computing the nonparametric and allele-sharing LOD scores specific to parental origins (LOD_{MO}, conditioned on maternal identity-by-descent [IBD] sharing, and LOD_{FA}, conditioned on paternal IBD sharing) among affected sibling pairs with an imprinting-based scoring function (Kong and Cox 1997; Gudbjartsson et al. 2000; Karason et al. 2003). The ALLEGRO program employs allele-sharing models developed by Kong and Cox (1997), which are closely related to nonparametric linkage scores. However, the parameter δ is maximized over both positive and negative values in ALLEGRO. The imprinting-based scoring function allows for calculation of parent-of-origin–specific allele sharing in the evaluation of linkage among affected sibling pairs (ASPs). We report genome-scan results based on sex-averaged genetic map distances. We also used sex-specific genetic maps from the Marshfield Genetic Map Database, to rerun the analyses for the EA sample, and got very similar results.

In analyses of the separate samples, frequencies were based on allele counting by use of all individuals in the sample. In the analysis of the combined samples, the corresponding allele sizes for the microsatellite markers were not available for the MB sample. However, all parental genotypes were available for the MB sample, so that allele-frequency estimates cannot affect the outcome of analyses. Alleles were matched between samples on the basis of frequency and, when necessary, were relabeled in the MB sample. This procedure resulted in identical allele frequencies for the MB sample. To examine the potential effect of allele-frequency estimates on the results, we ran the analyses for the individual samples using both the allele frequencies based on individual samples and those based on the combined sample and got very similar results. We reported the results in the analysis of combined data, using the frequencies based on allele counting with all individuals in the pooled sample.

To confirm results from the analyses that used ALLEGRO, we also used the ASPEX package to perform analyses of chromosome 10 data. The method employed in ASPEX is likelihood based and makes use of Risch's parameterization for relatives. We used the sib_ibd program in ASPEX to calculate the single-point allele-sharing proportion of paternal, maternal, and combined origin among ASPs. Similarly, we used sex-specific genetic maps to conduct multipoint analyses for male and female meioses separately by using a maximum-likelihood approach with the "most-likely" option of ASPEX.

To assess the statistical significance of the difference

Table 2

Regions with a Parent-Specific Single-Point LOD Score ≥2.0 for the EA Sample

Chromosome and Marker	Location (cM)	LOD_{MO}	LOD_{FA}	LOD	Phenotype
2:					
D2S2952	18	.00	2.00	1.11	Waist
D2S1400	28	.10	2.45	1.66	BMI
3:					
D3S2403	37	2.20	.00	.87	%fat
D3S3045	124	.00	3.66	1.79	BMI
9:					
D9S910	104	2.28	.57	2.42	BMI ⩾ 30
10:					
D10S197	52	2.71	.00	2.60	BMI ⩾ 27
11:					
D11S1993	54	.07	2.21	1.75	%fat
D11S2371	76	.02	2.00	1.18	%fat
12:					
D12S2070	125	2.32	.59	2.49	BMI
D12S2070	125	2.85	.17	2.04	Waist
13:					
D13S894	33	2.34	.10	1.57	BMI ⩾ 27
D13S793	76	.01	4.79	2.81	BMI
D13S793	76	.07	3.11	2.06	Waist

between maternal effect and paternal effect, we generated replicates through two approaches. One was to generate 20,000 replicates (no linkage) on the basis of the pedigree structure and marker allele frequency by using MERLIN and to perform linkage analyses by using ALLEGRO. Another was to generate 5,000 replicates by randomly assigning real parental genotypes of "mother" and "father" in the sibships (preserved linkage) and to perform analyses of replicates by using ALLEGRO. The latter analysis isolated the parent-of-origin effect from overall evidence of linkage. We calculated empirical *P* values (P_{MO}, P_{FA}, and $P_{LOD\,diff}$) based on the proportion of times that the observed LOD_{MO}, LOD_{FA}, and $|LOD_{MO} - LOD_{FA}|$ values for real data were equal to or exceeded by the LOD_{MO}, LOD_{FA}, and $|LOD_{MO} - LOD_{FA}|$ values, respectively, for each replicate at the specific marker.

Parent-Specific Linkage Analyses of Quantitative Traits

We used a family regression model to examine parent-of-origin effects on the three obesity-related quantitative traits by considering the parent-specific IBD-sharing proportion between sib pairs. We estimated parent-specific IBD-sharing proportion by using MERLIN and conducted the analyses by using a program written in SAS and based on the method presented by Sham et al. (2002) (L.L., O.Y.G., C.I.A., et al., unpublished data). This method regresses IBD sharing onto quantitative-trait differences and sums for sibling pairs. The regression method appears to be the most appropriate for selected samples such as ours.

As with the analyses of qualitative phenotypes, we generated replicates by two approaches. One was to generate 20,000 replicates by permutation of the observed IBD-sharing proportion between sib pairs within the sibships of the same size (no linkage). Another was to generate 5,000 replicates by randomly assigning real parent-specific IBD sharing to "maternal IBD" and "paternal IBD" in the sibships (preserved linkage). We calculated empirical *P* values (P_{MO}, P_{FA}, $P_{LOD\,diff}$, and $P_{Q\,diff}$) based on the proportion of times that the observed Q_{MO}, Q_{FA}, $|LOD_{MO} - LOD_{FA}|$, and $|Q_{MO} - Q_{FA}|$ values for real data were equal to or exceeded by the Q_{MO}, Q_{FA}, $|LOD_{MO} - LOD_{FA}|$, and $|Q_{MO} - Q_{FA}|$ values, respectively, for each replicate at the specific marker. *Q* is the phenotypic variance explained by the additive effects of the QTL in the regression model (Sham et al. 2002).

Results

Sib Pairs by Sample and Phenotype

Table 1 gives the number of sib pairs by sample and phenotype. For discrete traits, there were 658 and 472 ASPs with BMI ⩾ 27 and BMI ⩾ 30, respectively, in the EA sample. The corresponding numbers for the AA sample were 181 and 138 ASPs. In the MB sample, there were 116 ASPs with age- and sex-specific BMI percentile ⩾90th. For quantitative traits, the EA sample had 1,280 sib pairs with measured BMI, 1,103 with measured waist circumference, and 940 with measured %fat, whereas the AA sample had 323 sib pairs with BMI, 291 with waist circumference, and 284 with %fat.

Genomewide Screen for Parent-of-Origin Effects

Table 2 lists all regions with a LOD_{MO} or LOD_{FA} score ⩾2.0 for the discrete or quantitative traits in the genomewide single-point analysis of the EA sample. A maternal effect of $LOD_{MO} \geq 2.0$ was found in regions 3p (for %fat), 9q (for BMI ⩾ 30), 10p (for BMI ⩾ 27), 12q (for waist circumference and BMI), and 13q (for BMI ⩾ 27), whereas a paternal effect of $LOD_{FA} \geq 2.0$ was found in regions 2p (for BMI and waist circumference), 3q (for BMI), 11q (for %fat), and 13q (for BMI and waist circumference).

The multipoint LOD scores from the genome scan in the EA sample for discrete and quantitative traits are illustrated in figures 1 and 2, respectively. For discrete traits, the maximum LOD_{MO} score occurred at marker *D10S197* in the 10p12 region (LOD_{MO} = 2.42 for BMI ⩾ 27), whereas the maximum LOD_{FA} score appeared at 114 cM in 7q (LOD_{FA} = 1.69 for BMI ⩾ 27). For quantitative traits, LOD scores ⩾3.0 were found at marker *D12S2070* in region 12q24 (LOD_{MO} = 4.01 for BMI and 3.69 for waist circumference) and at marker *D13S793* in region 13q32 (LOD_{FA} = 3.72 for BMI) (fig. 3).

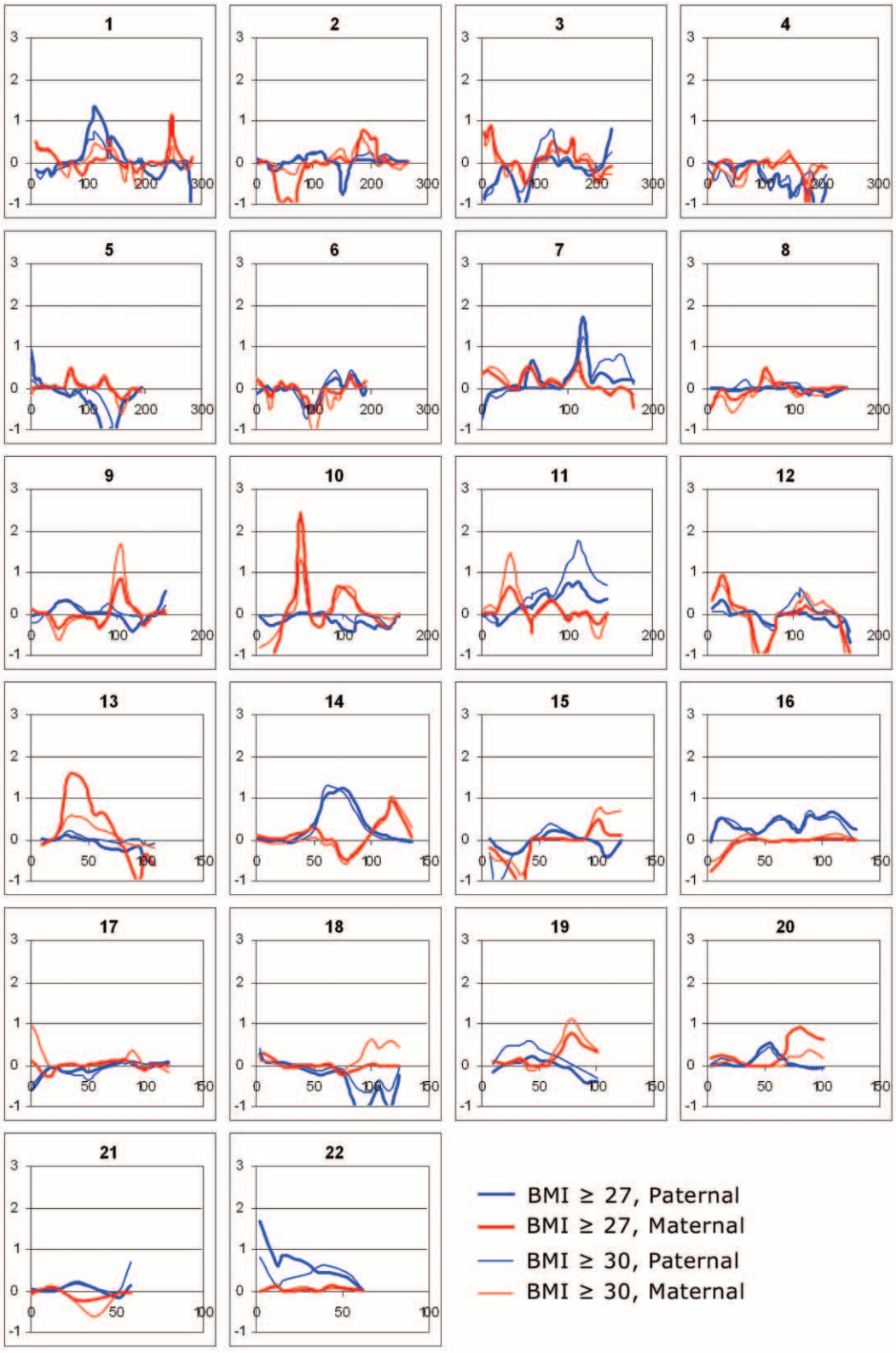

Figure 1 Results of genomewide parent-of-origin analyses for discrete traits (BMI ≥ 27 and BMI ≥ 30) in the EA sample. The *Y*-axis presents multipoint LOD scores, with consideration of only maternal transmission (*red line*) and only paternal transmission (*blue line*). Chromosome numbers are indicated above each graph.

Figure 2 Results of genomewide parent-of-origin analyses for quantitative traits (BMI, waist circumference, and %fat) in the EA sample. The *Y*-axis presents multipoint LOD scores, with consideration of only maternal transmission (*red line*) and only paternal transmission (*blue line*). Chromosome numbers are indicated above each graph.

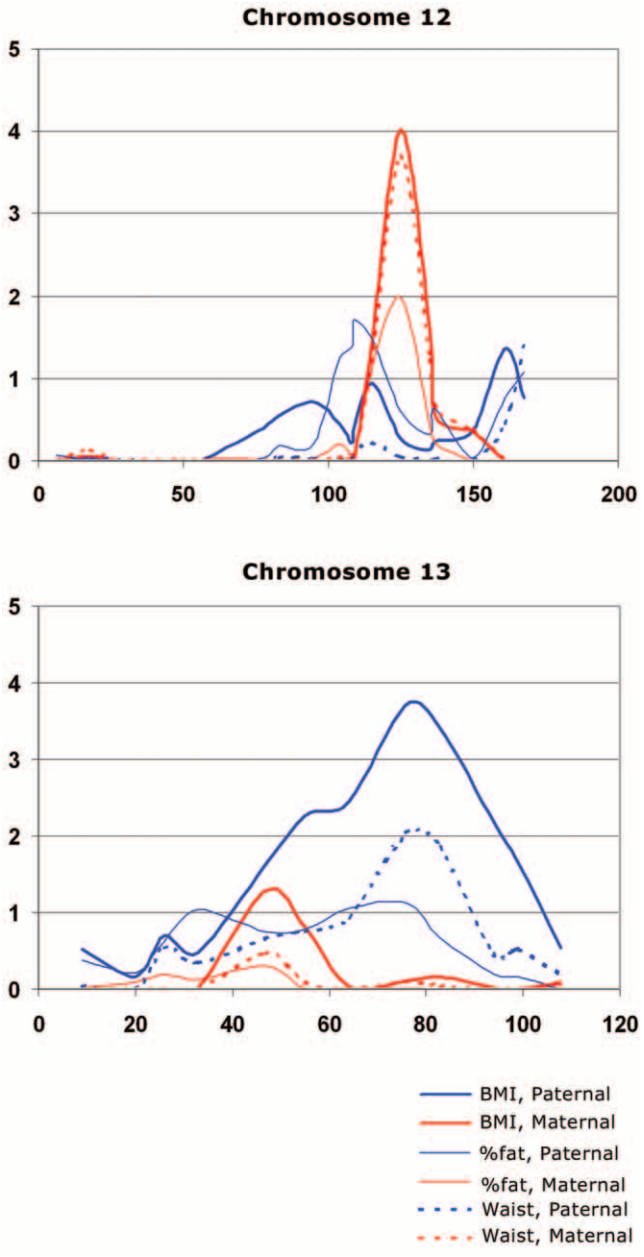

Figure 3 Results for chromosomes 12 and 13 for quantitative traits (BMI, waist circumference, and %fat) in the EA sample. The *Y*-axis presents multipoint LOD scores, with consideration of only maternal transmission (*red line*) and only paternal transmission (*blue line*).

Replicate and Combined Linkage Analyses of Chromosome 10

Among the regions with a parent-specific LOD score ≥2 in the EA sample, the only replicated result with a LOD_{MO} score >1.3 for a discrete trait in the single-point analysis was found at 10p12, across the three independent samples. At marker *D10S197*, the MB sample had a single-point LOD_{MO} of 1.86, versus a LOD_{FA} of 0.73, and the AA sample had a single-point LOD_{MO} of 1.31, versus a LOD_{FA} of 0.01. Table 3 summarizes parent-specific LOD scores for the three chromosome 10 markers that were common to the three samples—*D10S197*, *D10S193*, and *D10S1781*—for separate and combined data. When we combined the EA and AA samples, we obtained a single-point LOD_{MO} of 3.93, versus a LOD_{FA} of 0.01, and a multipoint LOD_{MO} of 3.65, versus a LOD_{FA} of 0.11, at marker *D10S197*. Similarly, when we combined the EA and MB samples, we obtained a single-point LOD_{MO} of 4.43, versus a LOD_{FA} of 0.28, and a multipoint LOD_{MO} of 3.28, versus a LOD_{FA} of 0.05, at marker *D10S197*. When we combined all three samples, we got a single-point LOD_{MO} of 5.69, versus a LOD_{FA} of 0.31, and a multipoint LOD_{MO} of 4.52, versus a LOD_{FA} of 0.10, at marker *D10S197*.

To verify the results for chromosome region 10p by use of different methods, we employed a maximum-likelihood model in ASPEX. The EA sample had a single-point LOD_{MO} of 2.26, versus a LOD_{FA} of −0.48, and a multipoint LOD_{MO} of 2.03, versus a LOD_{FA} of −0.23, at marker *D10S197*. At the same marker (*D10S197*), the MB sample had a single-point LOD_{MO} of 1.88, versus a LOD_{FA} of 0.43, and a multipoint LOD_{MO} of 1.70, versus a LOD_{FA} of 0.50, and the AA sample had a single-point LOD_{MO} of 1.11, versus a LOD_{FA} of −0.31, and a multipoint LOD_{MO} of 2.82, versus a LOD_{FA} of −0.88. For the combined EA and AA sample, at marker *D10S197*, we obtained a single-point LOD_{MO} of 3.38, versus a LOD_{FA} of −0.75, and a multipoint LOD_{MO} of 4.11, versus a LOD_{FA} of −0.78. For the pooled sample (EA+AA+MB), we obtained a single-point LOD_{MO} of 5.71, versus a LOD_{FA} of −0.57, and a multipoint LOD_{MO} of 4.71, versus a LOD_{FA} of −0.61, at marker *D10S197*. Figure 4 presents the multipoint linkage results for chromosome 10 by analysis of the individual samples (EA, AA, and MB) or combined samples (EA+AA+MB, EA+AA, and EA+MB).

Simulations in Assessment of Parent-of-Origin Effects at 10p12, 12q24, and 13q32

Table 4 presents the empiricial *P* values for markers *D10S197*, *D12S2070*, and *D13S793*. For the discrete trait BMI ≥ 27 and the pooled sample (EA+AA+MB), the empirical *P* value for $|LOD_{MO} - LOD_{FA}|$ at marker *D10S197* was <.00005, on the basis of 20,000 simulations under no linkage, and was .0126, on the basis of 5,000 simulations under the model that preserved overall linkage. For BMI and waist circumference in the EA sample, the empirical *P* value at 12q marker *D12S2070* was <.00005 for $|LOD_{MO} - LOD_{FA}|$ and was <.025 for $|Q_{MO} - Q_{FA}|$, on the basis of 20,000 simulations under no linkage, and all corresponding empirical *P* values were <.05, on the basis of 5,000 sim-

Table 3

Parent-Specific Linkage LOD Scores at Markers *D10S197, D10S193,* and *D10S1781,* for Discrete Traits

Sample and Marker	Location (cM)	Single-Point		Multipoint	
		LOD_{MO}	LOD_{FA}	LOD_{MO}	LOD_{FA}
EA+AA+MB:					
D10S197	52	5.69	.31	4.52	.10
D10S193	59	1.53	.00	2.65	.04
D10S1781	61	1.44	.30	2.72	.11
EA+MB:					
D10S197	52	4.43	.28	3.28	.05
D10S193	59	.93	.02	2.11	.07
D10S1781	61	1.27	.30	2.08	.13
EA+AA:					
D10S197	52	3.93	.01	3.65	.11
D10S193	59	.74	.00	1.91	.03
D10S1781	61	.68	.16	1.66	.06
EA:					
D10S197	52	2.71	.00	2.33	.03
D10S193	59	.27	.02	1.28	.05
D10S1781	61	.51	.16	1.12	.06
MB:					
D10S197	52	1.86	.73	.71	.15
D10S193	59	.91	.00	1.03	.08
D10S1781	61	1.01	.15	1.40	.15
AA:					
D10S197	52	1.31	.01	1.35	.09
D10S193	59	.77	.13	.56	.02
D10S1781	61	.12	.00	.46	.01

Note.—Discrete traits were BMI $\geq$ 27 for the EA and AA samples and BMI $\geq$90th percentile for the MB sample. Scores were calculated using ALLEGRO.

ulations under preserved overall linkage. The empirical P values for $|LOD_{MO} - LOD_{FA}|$ at 13q marker *D13S793* were .00005 for BMI, .00145 for waist circumference, and .01715 for %fat, on the basis of 20,000 simulations under no linkage. However, the empirical $P < .05$ at marker *D13S793* was limited to BMI for $|Q_{MO} - Q_{FA}|$.

Discussion

Our linkage results suggest that there are at least three genetic loci—in chromosome regions 10p12, 12q24, and 13q32—that may influence susceptibility to obesity when it is maternally or paternally transmitted. None of the locations correspond to regions known to harbor imprinted genes.

To date, ~14 regions on 10 chromosomes have shown evidence or proof of imprinting in humans, including at least 26 paternally expressed genes and 13 maternally expressed genes, and at least 20 genetic diseases show proof of imprinting (Nicholls 2000; Hayward et al. 2001; Lalande 2001; Shore et al. 2002). Currently, no imprinted genes on chromosomes 10p12, 12q22-24, and 13q32 have been identified. The chromosome 10 linkage is at its maximum at marker *D10S197*, which is in intron 7 of the gene for glutamic acid decarboxylase 2 (*GAD2*). Variation in this gene has been associated with obesity (Boutin et al. 2003), but the gene is not known to be imprinted, nor are there other genes nearby that are currently known to be imprinted. No specific gene variation has been associated with obesity-related traits in the regions identified on chromosomes 12 and 13, and no currently known imprinted genes are located in close proximity. Although the number of genes in these regions is large, it may be possible to substantially reduce the number of potential candidate genes *in silico* by use of predictive models (Greally 2002). The smaller set could be evaluated using the orthologous genes in mouse models.

Although the parent-of-origin effect detected on chromosome 7 did not meet our threshold of a LOD score $\geq$2, it lies in a region for which we have reported linkage (Li et al. 2003). The linkage is interesting because the location (~108 Mb) is directly between two regions on 7q (~95 Mb and ~130 Mb) that are known to have blocks of imprinted genes (Okita et al. 2003). The full extent of these two regions is unknown. Given its location between these two imprinted blocks, this secondary linkage will be interesting to examine in more detail in other studies.

Although it has been estimated that only ~1% of the human genome is imprinted, a common theory concerning imprinting is that it arose in mammals because of differential parental investment in body size. In fact, many imprinted genes have been associated with growth and behavior (Nicholls 2000; Keverne 2001). For example, the imprinted genes *IGF2* and *insulin* are embryonic growth enhancers (paternally expressed), whereas the maternally expressed *Igf2r* and *p57KIP2* genes inhibit embryonic growth. Other imprinted genes, such as *Rasgrf* in mouse (Itier et al. 1998) and the genes in the region of Prader-Willi syndrome (MIM 176270), are paternally expressed genes that regulate postnatal growth, particularly in the neonatal period (Nicholls and Knepper 2001). Furthermore, several imprinted human disorders show abnormal embryonic or neonatal growth and/or changes in body composition, including Beckwith-Wiedemann syndrome (MIM 130650), Prader-Willi syndrome, Angelman syndrome (MIM 105830), neonatal diabetes, Russell-Silver syndrome (MIM 312780), and Albright hereditary osteodystrophy (MIM 103580), as well as the related conditions of progressive osseous heteroplasia, acromegaly, and pseudohypoparathyroidism types 1a and 1b (MIM 603233) (Nicholls 2000; Hayward et al. 2001; Lalande 2001; Shore et al. 2002).

Studies in mouse models and agricultural animals further support a role for imprinted genes in body composition. For example, paternal transmission but not maternal transmission of a gene knockout of *Pref1/Dlk1*

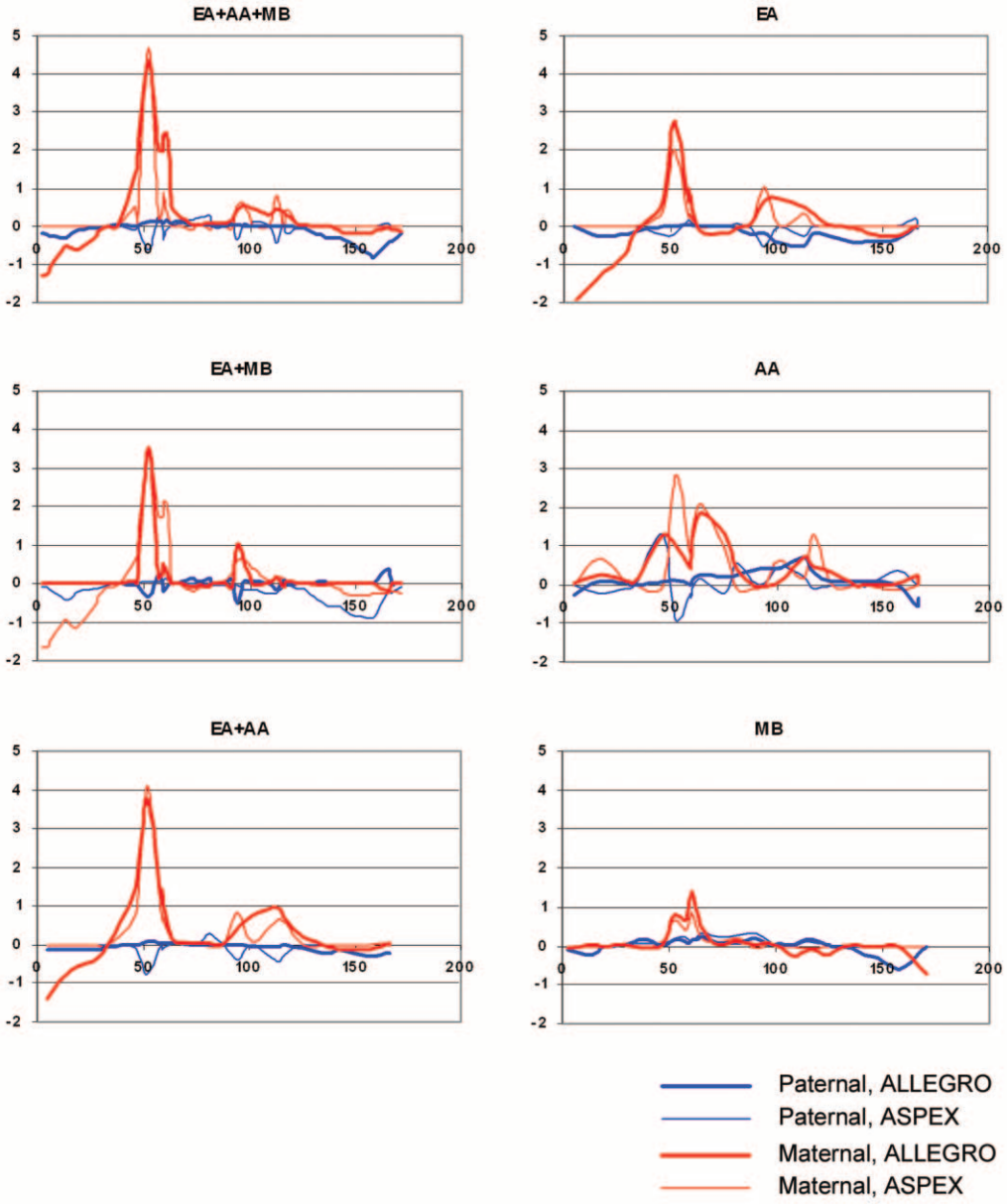

Figure 4 Results for chromosome 10 in analyses of discrete traits (BMI ⩾ 27 in the EA and AA samples; BMI ⩾90th percentile in the MB sample) in the individual and combined samples, by use of ALLEGRO and ASPEX. The *Y*-axis presents multipoint LOD scores, with consideration of only maternal transmission (*red line*) and only paternal transmission (*blue line*).

leads to neonatal growth retardation and subsequent increased adiposity (Moon et al. 2002). This phenotype is similar to the callipyge mutation at the homologous locus in sheep, for which there is a phenotype of increased muscling and decreased adipose tissue as a consequence of abnormally regulated expression of multiple imprinted genes within an imprinted domain (Charlier et al. 2001).

Recently, several studies have searched for QTLs affecting body composition in pigs, including back-fat thickness, muscle depth, and intramuscular fat content. Surprisingly, in one study (de Koning et al. 2000), four of five QTLs showed evidence of a parental-origin effect, with two loci consistent with a paternally expressed locus and two others consistent with a maternally expressed locus. Indeed, a QTL affecting body composition in pigs maps to the region harboring *Igf2*, a paternally expressed gene (Jeon et al. 1999). These observations suggest that loci associated with body composition might frequently be regulated by a parent-

Table 4

Empirical *P* Values for Markers *D10S197, D12S2070,* and *D13S793*

Marker (Sample) and Phenotype	Empirical *P* Values			
	P_{MO}	P_{FA}	$P_{LOD\,diff}$	$P_{Q\,diff}$
D10S197 (EA+AA+MB):				
BMI ⩾ 27[a]	<.00005	.10980	<.00005	…
BMI ⩾90th percentile[a]	(.0056)	(.9912)	(.0126)	…
D12S2070 (EA):				
BMI[b]	.00005 (.0174)	.13225 (.9794)	<.00005 (.0404)	.02235 (.0390)
Waist circumference[b]	.00005 (.0086)	.31760 (.9864)	<.00005 (.0222)	.00780 (.0212)
%fat[b]	.00205 (.1599)	.05045 (.8091)	.01115 (.3946)	.29420 (.3475)
D13S793 (EA):				
BMI[b]	.22285 (.9942)	.00005 (.0182)	.00005 (.0152)	.02150 (.0196)
Waist circumference[b]	.24400 (.9396)	.00175 (.1056)	.00145 (.1404)	.11140 (.1644)
%fat[b]	.38045 (.9040)	.00805 (.0598)	.01715 (.1536)	.15050 (.1494)

[a] For discrete traits, P_{MO}, P_{FA}, and $P_{LOD\,diff}$ were based on the proportion of times that the observed LOD_{MO}, LOD_{FA}, and $|LOD_{MO} - LOD_{FA}|$ values for real data were equal to or exceeded by the LOD_{MO}, LOD_{FA}, and $|LOD_{MO} - LOD_{FA}|$ values, respectively, for each replicate at the specific marker. Empirical *P* values not in parentheses were based on 20,000 replicates generated by MERLIN (no linkage). Empirical *P* values in parentheses were based on 5,000 replicates generated by randomly assigning the real parental genotypes to "mother" or "father" in the sibships (preserved overall linkage).

[b] For quantitative traits, P_{MO}, P_{FA}, $P_{LOD\,diff}$, and $P_{Q\,diff}$ were based on the proportion of times that the observed Q_{MO}, Q_{FA}, $|LOD_{MO} - LOD_{FA}|$, and $|Q_{MO} - Q_{FA}|$ values for real data were equal to or exceeded by the Q_{MO}, Q_{FA}, $|LOD_{MO} - LOD_{FA}|$, and $|Q_{MO} - Q_{FA}|$ values, respectively, for each replicate at the specific marker. Empirical *P* values not in parentheses were based on 20,000 replicates generated by permutation (no linkage). Empirical *P* values in parentheses were based on 5,000 replicates generated by randomly assigning the real parent-specific IBD to "maternal IBD" or "paternal IBD" in the sibships (preserved overall linkage).

of-origin phenomenon, such as genomic imprinting. Furthermore, they provide support for the linkage-based approach we have taken in determining whether imprinted genes play a role in complex human traits such as obesity.

Only three studies of obesity-related traits in humans have incorporated parent-of-origin effects. Lindsay et al. (2001) used a variance-components approach developed by Amos (1994) that was modified to calculate LOD scores by parent of origin, to examine BMI in Pima Indians. Significance was tested using a likelihood-ratio test for differences in maternal and paternal LOD scores. Lindsay et al. (2001) reported suggestive linkage to regions of chromosome 5 (67–75 cM; maternal LOD = 1.7) and chromosome 10 (6–20 cM; paternal LOD = 1.7). Lindsay et al. (2002) also examined birth weight by use of similar methods and found a multipoint paternal LOD score of 3.1, with a peak at 88 cM on chromosome 11, far from the known imprinted region at the p telomere. The maternal contribution was zero. Gorlova et al. (2003) used an extension of methods developed by Amos (1994) that incorporated parent-of-origin effects (Shete and Amos 2002). They examined BMI in samples of children (aged 5–11 years), adolescents (aged 12–16 years), and young adults (aged 17–30 years). Parent-of-origin effects were found in the youngest sample for chromosomes 3 (45 cM), 4 (158 cM), 10 (46 cM), and 12 (18 cM). No linkage was found in the adolescent group. Effects were found in the oldest group for chromosomes 4 (182 cM) and 8 (8 cM). None of the linkages were found in any two of the three samples. The lack of replication could have been due to differences in the ethnic groups examined (e.g., Pima Indians [Lindsay et al. 2001, 2002] and European Americans [Gorlova et al. 2003]), phenotype (BMI and birth weight in the Pima sample and BMI in the EA sample), age (Lindsay et al. 2001, 2002; Gorlova et al. 2003), or the small size of the EA sample (Gorlova et al. 2003).

Several independent linkage studies have suggested the existence of obesity-predisposition loci in chromosome region 10p (Hager et al. 1998; Hinney et al. 2000; Hsueh et al. 2001; Price et al. 2001). One candidate gene for obesity (*GAD2*) is found in this region (Boutin et al. 2003); however, the gene is not known to be imprinted. The consistency of results across three samples in our study suggest that *GAD2* could be imprinted, at least in some tissues at certain times during development. It is also possible that the maternal effect is due to other genes found in the region, since variability in the *GAD2* gene did not account for the observed linkage in a French sample (Boutin et al. 2003).

In addition to 10p12, 12q22-24, and 13q32, we observed several regions with parent-of-origin–specific linkage in the EA sample. For example, we obtained a single-point LOD_{FA} score of 2.21 (LOD_{MO} = 0.07

for %fat) at 76 cM on chromosome 11q13 near two candidate genes for obesity (*UCP2* and *UCP3*) (Snyder et al. 2004) and an imprinted gene for hereditary paraganglioma (*SDHD,* with paternal inheritance) (Struycken et al. 1997). We obtained a single-point LOD_{MO} score of 2.34 (LOD_{FA} = 0.11 for BMI ⩾27) at 33 cM on chromosome 13q near another candidate gene for obesity (*HTR2A,* an imprinted gene for retinoblastoma, with maternal inheritance) (Kato et al. 1998).

Differences between results of analyses of quantitative versus discrete traits are common, as was the case in the present study. Ultimately, differences result from the different aspects of the data that are the focus of the analyses. Discrete-trait analyses focus on IBD in ASPs, whereas quantitative analyses focus on the entire trait distribution, often including unaffected siblings, parents, and other relatives. In this particular case, we have hypothesized that differences may be the result of the presence of a wide range of phenotypes for any given genotype at a particular QTL. Chromosome 10p linkage results have consistently been found only by including individuals with moderate BMI in families segregating extreme obesity. Thus, the sampling of extremely obese individuals and those of normal weight appears to have selected for families segregating obesity-related genes residing on chromosome 10p. The range of phenotypes, we believe, may be influenced by interactions of the segregating genes with genetic background and environmental conditions such as lifestyle (gene-gene and gene-environment interactions).

Conclusion

We conducted a genomewide screen for parent-of-origin effects at genetic loci affecting susceptibility to obesity. The results from the original sample and two independent replicates were consistent in suggesting that a maternally imprinted genetic locus in chromosome region 10p12 may influence human obesity. We also found evidence of a maternal effect on chromosome 12 and a paternal effect on chromosome 13. A suggestive paternal effect found on chromosome 7q lies in a region flanked by blocks of imprinted genes. The other locations are not currently known to be imprinted. In addition to the direct relevance of these results to obesity, the findings suggest new locations within the human genome in which a search for imprinted genes may be productive.

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Electronic-Database Information

The URLs for data presented herein are as follows:

Marshfield Center for Medical Genetics, http://research.marshfieldclinic.org/genetics/ (for genetic map database and map locations)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for obesity, Prader-Willi syndrome, Beckwith-Wiedemann syndrome, Angelman syndrome, Russell-Silver syndrome, Albright hereditary osteodystrophy, and pseudohypoparathyroidism)

References

Amos CI (1994) Robust variance-components approach for assessing genetic linkage in pedigrees. Am J Hum Genet 54: 535–543

Boutin P, Dina C, Vasseur F, Dubois SS, Corset L, Seron K, Bekris L, Cabellon J, Neve B, Vasseur-Delannoy V, Chikri M, Charles MA, Clement K, Lernmark A, Froguel P (2003) *GAD2* on chromosome 10p12 is a candidate gene for human obesity. PLoS Biol 1:E68

Carroll KK (1998) Obesity as a risk factor for certain types of cancer. Lipids 33:1055–1059

Charlier C, Segers K, Karim L, Shay T, Gyapay G, Cockett N, Georges M (2001) The callipyge mutation enhances the expression of coregulated imprinted genes in *cis* without affecting their imprinting status. Nat Genet 27:367–369

de Koning DJ, Rattink AP, Harlizius B, van Arendonk JA, Brascamp EW, Groenen MA (2000) Genome-wide scan for body composition in pigs reveals important role of imprinting. Proc Natl Acad Sci USA 97:7947–7950

Gorlova OY, Amos CI, Wang NW, Shete S, Turner ST, Boerwinkle E (2003) Genetic linkage and imprinting effects on body mass index in children and young adults. Eur J Hum Genet 11:425–432

Greally JM (2002) Short interspersed transposable elements (SINEs) are excluded from imprinted regions in the human genome. Proc Natl Acad Sci USA 99:327–332

Grilo CM, Pogue-Geile MF (1991) The nature of environmental influences on weight and obesity: a behavior genetic analysis. Psychol Bull 110:520–537

Gudbjartsson DF, Jonasson K, Frigge ML, Kong A (2000) Allegro, a new computer program for multipoint linkage analysis. Nat Genet 25:12–13

Hager J, Dina C, Francke S, Dubois S, Houari M, Vatin V, Vaillant E, Lorentz N, Basdevant A, Clement K, Guy-Grand B, Froguel P (1998) A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. Nat Genet 20:304–308

Hayward BE, Barlier A, Korbonits M, Grossman AB, Jacquet P, Enjalbert A, Bonthron DT (2001) Imprinting of the $G_s\alpha$

gene *GNAS1* in the pathogenesis of acromegaly. J Clin Invest 107:R31–R36

Hinney A, Ziegler A, Oeffner F, Wedewardt C, Vogel M, Wulftange H, Geller F, Stubing K, Siegfried W, Goldschmidt HP, Remschmidt H, Hebebrand J (2000) Independent confirmation of a major locus for obesity on chromosome 10. J Clin Endocrinol Metab 85:2962–2965

Hsueh WC, Mitchell BD, Schneider JL, St Jean PL, Pollin TI, Ehm MG, Wagner MJ, Burns DK, Sakul H, BellCJ, Shuldiner AR (2001) Genome-wide scan of obesity in the Old Order Amish. J Clin Endocrinol Metab 86:1199–1205

Itier JM, Tremp GL, Leonard JF, Multon M, Ret G, Schweighoffer F, Tocque B, Bluet-Pajot MT, Cormier V, Dautry F (1998) Imprinted gene in postnatal growth. Nature 393:125–126

Jeon JT, Carlborg O, Törnsten A, Giuffra E, Amarger V, Chardon P, Andersson-Eklund L, Andersson K, Hansson I, Lundström K, Andersson L (1999) A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the *IGF2* locus. Nat Genet 21:157–158

Karason A, Gudjonsson JE, Upmanyu R, Antonsdottir AA, Hauksson VB, Runasdottir EH, Jonsson HH, Gudbjartsson DF, Frigge ML, Kong A, Stefansson K, Valdimarsson H, Gulcher JR (2003) A susceptibility gene for psoriatic arthritis maps to chromosome 16q: evidence for imprinting. Am J Hum Genet 72:125–131

Kato MV, Ikawa Y, Hayashizaki Y, Shibata H (1998) Paternal imprinting of mouse serotonin receptor 2A gene *Htr2* in embryonic eye: a conserved imprinting regulation on the *RB/Rb* locus. Genomics 47:146–149

Keverne EB (2001) Genomic imprinting, maternal care, and brain evolution. Horm Behav 40:146–155

Kong A, Cox NJ (1997) Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61:1179–1188

Kopelman PG (2000) Obesity as a medical problem. Nature 404:635–643

Lalande M (2001) Imprints of disease at *GNAS1*. J Clin Invest 107:793–794

Lee JH, Reed DR, Li W-D, Xu W, Joo E-J, Kilker RL, Nanthakumar E, North M, Sakul H, Bell C, Price RA (1999) Genome scan for human obesity and linkage to markers in 20q13. Am J Hum Genet 64:196–209

Li WD, Dong C, Li D, Zhao H, Price RA (2004) An obesity-related locus in chromosome region 12q23-24. Diabetes 53: 812–820

Li WD, Li D, Wang S, Zhang S, Zhao H, Price RA (2003) Linkage and linkage disequilibrium mapping of genes influencing human obesity in chromosome region 7q22.1-7q35. Diabetes 52:1557–1561

Lindsay RS, Kobes S, Knowler WC, Bennett PH, Hanson RL (2001) Genome-wide linkage analysis assessing parent-of-origin effects in the inheritance of type 2 diabetes and BMI in Pima Indians. Diabetes 50:2850–2857

Lindsay RS, Kobes S, Knowler WC, Hanson RL (2002) Genome-wide linkage analysis assessing parent-of-origin effects in the inheritance of birth weight. Hum Genet 110:503–509

Maes HH, Neale MC, Eaves LJ (1997) Genetic and environmental factors in relative body weight and human adiposity. Behav Genet 27:325–351

Moon YS, Smas CM, Lee K, Villena JA, Kim KH, Yun EJ, Sul HS (2002) Mice lacking paternally expressed Pref-1/Dlk1 display growth retardation and accelerated adiposity. Mol Cell Biol 22:5585–5592

Nicholls RD (2000) The impact of genomic imprinting for neurobehavioral and developmental disorders. J Clin Invest 105:413–418

Nicholls RD, Knepper JL (2001) Genome organization, function, and imprinting in Prader-Willi and Angelman syndromes. Annu Rev Genomics Hum Genet 2:153–175

Okita C, Meguro M, Hoshiya H, Haruta M, Sakamoto YK, Oshimura M (2003) A new imprinted cluster on the human chromosome 7q21-q31, identified by human-mouse monochromosomal hybrids. Genomics 81:556–559

Price RA (2001) Genetics and common obesities: background, current status, strategies and future prespects. In: Wadden TS (ed) Obesity, theory and therapy. Guilford Publications, New York

Price RA, Gottesman II (1991) Body fat in identical twins reared apart: roles for genes and environment. Behav Genet 21:1–7

Price RA, Li WD, Bernstein A, Crystal A, Golding EM, Weisberg SJ, Zuckerman WA (2001) A locus affecting obesity in human chromosome region 10p12. Diabetologia 44:363–366

Price RA, Reed DR, Lee JH (1998) Obesity related phenotypes in families selected for extreme obesity and leanness. Int J Obes Relat Metab Disord 22:406–413

Sham PC, Purcell S, Cherny SS, Abecasis GR (2002) Powerful regression-based quantitative-trait linkage analysis of general pedigrees. Am J Hum Genet 71:238–253

Shete S, Amos CI (2002) Testing for genetic linkage in families by a variance-components approach in the presence of genomic imprinting. Am J Hum Genet 70:751–757

Shmulewitz D, Auerbach SB, Lehner T, Blundell ML, Winick JD, Youngman LD, Skilling V, Heath SC, Ott J, Stoffel M, Breslow JL, Friedman JM (2001) Epidemiology and factor analysis of obesity, type II diabetes, hypertension, and dyslipidemia (syndrome X) on the Island of Kosrae, Federated States of Micronesia. Hum Hered 51:8–19

Shore EM, Ahn J, Jan de Beur S, Li M, Xu M, Gardner RJ, Zasloff MA, Whyte MP, Levine MA, Kaplan FS (2002) Paternally inherited inactivating mutations of *GNAS1* gene in progressive osseous heteroplasia. N Engl J Med 346:99–106

Snyder EE, Walts B, Perusse L, Chagnon YC, Weisnagel SJ, Rankinen T, Bouchard C (2004) The human obesity gene map: the 2003 update. Obes Res 12:369–439

Sorensen TI, Price RA, Stunkard AJ, Schulsinger F (1989) Genetics of obesity in adult adoptees and their biological siblings. BMJ 298:87–90

Struycken PM, Cremers CW, Mariman EC, Joosten FB, Bleker RJ (1997) Glomus tumours and genomic imprinting: influence of inheritance along the paternal or maternal line. Clin Otolaryngol 22:71–76

Stunkard AJ, Harris JR, Pedersen NL, McClearn GE (1990) The body-mass index of twins who have been reared apart. N Engl J Med 322:1483–1487

Stunkard AJ, Sorensen TI, Hanis C, Teasdale TW, Chakraborty R, Schull WJ, Schulsinger F (1986) An adoption study of human obesity. N Engl J Med 314:193–198

Wolk A, Gridley G, Svensson M, Nyren O, McLaughlin JK, Fraumeni JF, Adam HO (2001) A prospective study of obesity and cancer risk (Sweden). Cancer Causes Control 12:13–21