Sex-Limited Genome-Wide Linkage Scan for Body Mass Index in an Unselected Sample of 933 Australian Twin Families

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Genes involved in pathways regulating body weight may operate differently in men and women. To determine whether sex-limited genes influence the obesity-related phenotype body mass index (BMI), we have conducted a general non-scalar sex-limited genome-wide linkage scan using variance components analysis in Mx (Neale, 2002). BMI measurements and genotypic data were available for 2053 Australian female and male adult twins and their siblings from 933 families. Clinical measures of BMI were available for 64.4% of these individuals, while only self-reported measures were available for the remaining participants. The mean age of participants was 39.0 years of age (*SD* 12.1 years). The use of a sex-limited linkage model identified areas on the genome where quantitative trait loci (QTL) effects differ between the sexes, particularly on chromosome 8 and 20, providing us with evidence that some of the genes responsible for BMI may have different effects in men and women. Our highest linkage peak was observed at 12q24 ($-\log_{10}p$ = 3.02), which was near the recommended threshold for suggestive linkage ($-\log_{10}p$ = 3.13). Previous studies have found evidence for a quantitative trait locus on 12q24 affecting BMI in a wide range of populations, and candidate genes for non-insulin-dependent diabetes mellitus, a consequence of obesity, have also been mapped to this region. We also identified many peaks near a $-\log_{10}p$ of 2 (threshold for replicating an existing finding) in many areas across the genome that are within regions previously identified by other studies, as well as in locations that harbor genes known to influence weight regulation.

The prevalence of obesity has reached epidemic proportions with the percentage of people overweight or obese doubling in the last 30 years. In 2000 it was estimated that 48% of Australian adult men were overweight and 19% were obese, and 52% of adult females were either overweight (31%) or obese (21%; Dunstan et al., 2001). Additionally, almost a quarter of Australian children and adolescents are too heavy (Australian Institute of Health and Welfare, 2000; Eckersley, 2003). Obesity is a major risk factor for numerous life-threatening diseases including Type II diabetes, hypertension, stroke, coronary heart disease and cancer including breast, prostate and colon cancers (Hill et al., 2003; Marx, 2003; Pi-Sunyer, 2002; Sibbald, 2002). The substantial increase in health risks has made obesity the second leading cause of death behind smoking (Sibbald, 2002).

Studies of weight in humans commonly use the obesity-related phenotype body mass index (BMI) as it is very easy to measure in large epidemiological studies. BMI is calculated as weight(kg)/height(m)^2 and is highly correlated with direct measures of fat mass in both adults ($r \approx .8$) and adolescents ($r \approx .6$; Borecki et al., 1998; Feitosa et al., 2002).

Results from studies of twins reared apart and together have suggested that genetic effects explain 50% to 90% of the variation in BMI (Dong et al., 2003). However, recent increases in the prevalence of obesity are likely to have been caused by changes in the environment rather than changes in genetic background, given the short period over which this increase has occurred. The cultural environment of most western societies encourages the consumption of energy and discourages its expenditure, in direct contrast to our hunter–gatherer past. Identifying genes that are maladaptive to our current sedentary lifestyle of overabundance may

provide a better understanding of the genes that influence the susceptibility to weight gain.

Sex-by-genotype interactions may further complicate the investigation of genes that are responsible for the development of obesity. Body composition differs between the sexes, with fat distribution tending to be more abdominal in men and pelvic in women. Numerous studies document that there is gender-specific variation in BMI. Two twin studies on 16- to 17-year-old twins (Pietilainen et al., 1999) and 18- to 25-year-old twins (Harris et al., 1995) reported that the sets of genes influencing variation in BMI are distinct in males and females. Analyses of Australian twin data have produced mixed results. An analysis of BMI in a twin sample split into two cohorts (aged 18 to 30 years and 31 years and above) found no evidence for sex differences, although different models were obtained for the two age cohorts (Neale & Cardon, 1992). However, a subsequent analysis of a larger sample of Australian twins, including the original sample, did provide evidence of sex differences in the variation of BMI (Schousboe et al., 2003). In both the younger (aged 20 to 29 years) and older (aged 30 to 39 years) cohorts of this sample there was evidence for sex differences in the magnitude of genetic and environmental influences. Additionally, sex-specific genetic effects on variation in BMI were identified, although only in the younger cohort. In a Caucasian and African–American population, some genetic effects on BMI were distinct in men and women, while the magnitude of these effects was similar in both sexes (Lewis et al., 2005).

The identification of genes responsible for common forms of obesity continues to be difficult. However, linkage analyses conducted on BMI have consistently identified regions located on chromosomes 7 (Clement et al., 1998; Duggirala et al., 1996; Feitosa et al., 2002; Heijmans et al., 2004; Lapsys et al., 1997; Reed et al., 1996; Roth et al., 1997), 10 (Deng et al., 2002; Hunt et al., 2001; Lee, 1999; Norman et al., 1998), 13 (Hager et al., 1998; Hinney et al., 2000; Price et al., 2001) and 20 (Feitosa et al., 2002; Lee, 1999). Furthermore, other findings suggest the influence of genes located on chromosomes 3 (Hsueh et al., 2001), 4 (Deng et al., 2002; Stone et al., 2002), 5 (Clement et al., 1999; Perola et al., 2001) and 11 (Atwood et al., 2002; Hanson et al., 1998) may play a role in the development of obesity. Only recently has there been investigation into possible sex differences in quantitative trait loci (QTLs) influencing BMI. Lewis et al. (2005) found that QTLs affecting another obesity-related phenotype, percentage body fat (PBF), were located in different areas of the genome in males and females. This QTL was located on 12p in women and on 15q in men. However, apart from this recent study, to our knowledge there have been no other studies published reporting different QTLs influencing BMI in males and females. Thus, in this article, not only have we examined genetic and environmental influences on BMI and how these might differ between males and females, we have also incorpo-rated these differences into genome-wide linkage analysis to test whether the magnitude of QTL effects differs between males and females.

Materials and Methods

Phenotype Data Collection and Cleaning

Clinical and self-reported height and weight data were derived from several studies of adult twins and their families recruited from the Australian Twin Registry. Self-reported measures were taken from Health and Lifestyle questionnaires sent out to twins, parents, siblings, spouses and children of the twins between 1980 and 1996 (Healey et al., 2001; Kirk, Bailey, et al., 2000), while clinical measurements were taken in the context of studies conducted in the years 1992 to 1996 (Schousboe et al., 2003; Silventoinen et al., 2003).

Most participants were involved in more than one study and therefore a number of individuals had several measurements of BMI. As a result, rules were implemented to clean the data. Through the participation in a variety of self-report studies, 16.7% ($N = 5124$) of these individuals attended a clinical measurement in which height and weight were measured by a nurse using a stadiometer and accurate scales respectively. If no clinical measurement existed, self-reported height and weight were analyzed (83.3%, $N = 25,521$). BMI discrepancies over time and extreme measures were checked carefully against original records and those which differed more than 2kg/m^2 from other BMI measures across studies were not included in any analyses. A detailed outline of these rules to determine which of the multiple BMI measures was used is specified in Figure 1. In total, data for height and weight phenotypes were available for 30,645 individuals; 7895 twin pairs, 1461 single twins, 3895 siblings, 1672 children of twins, 3521 parents of twins, and 4306 spouses of twins.

Genotype Data

The genotypic data used for this research represents a compilation from four smaller genome scans undertaken for particular phenotypic studies at the Queensland Institute of Medical Research. Recruitment of participants for these studies was primarily based upon participant involvement in previous phenotype collection studies. As a result, some individuals have been genotyped in more than one genome scan and have phenotypes available from more than one study. To maximize the sample and informativeness of the data available for linkage analyses, we combined the data from all four genome scans.

Individual Genotyping Datasets

In this section, we describe family ascertainment and genotyping as well as error checking performed prior to merging the four datasets. The recruitment relationships between the various studies and the study overlap of participants is represented in Figures 2 and 3, with the details of the studies including a genotyping component described below. Table 1
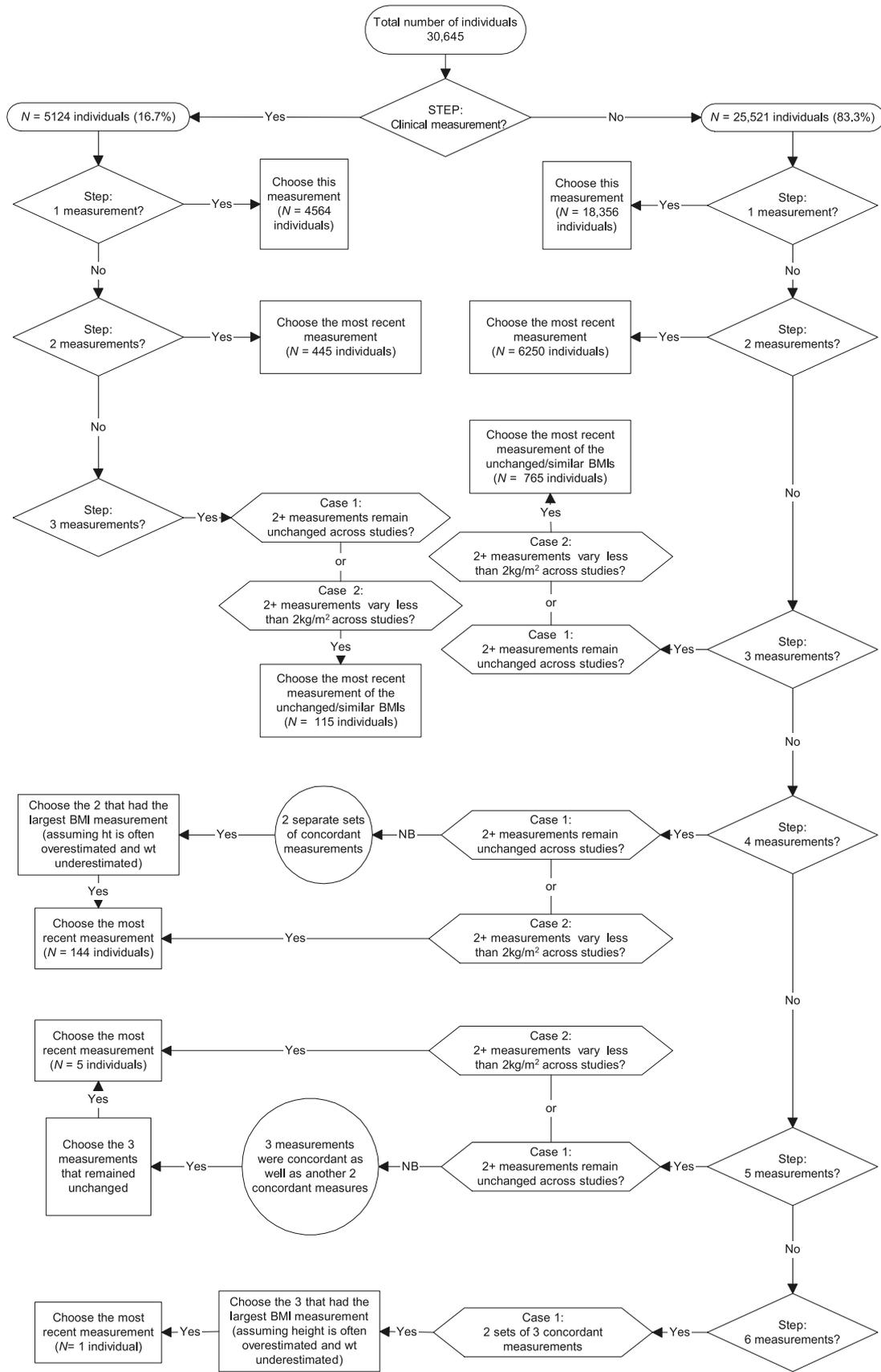
Figure 1

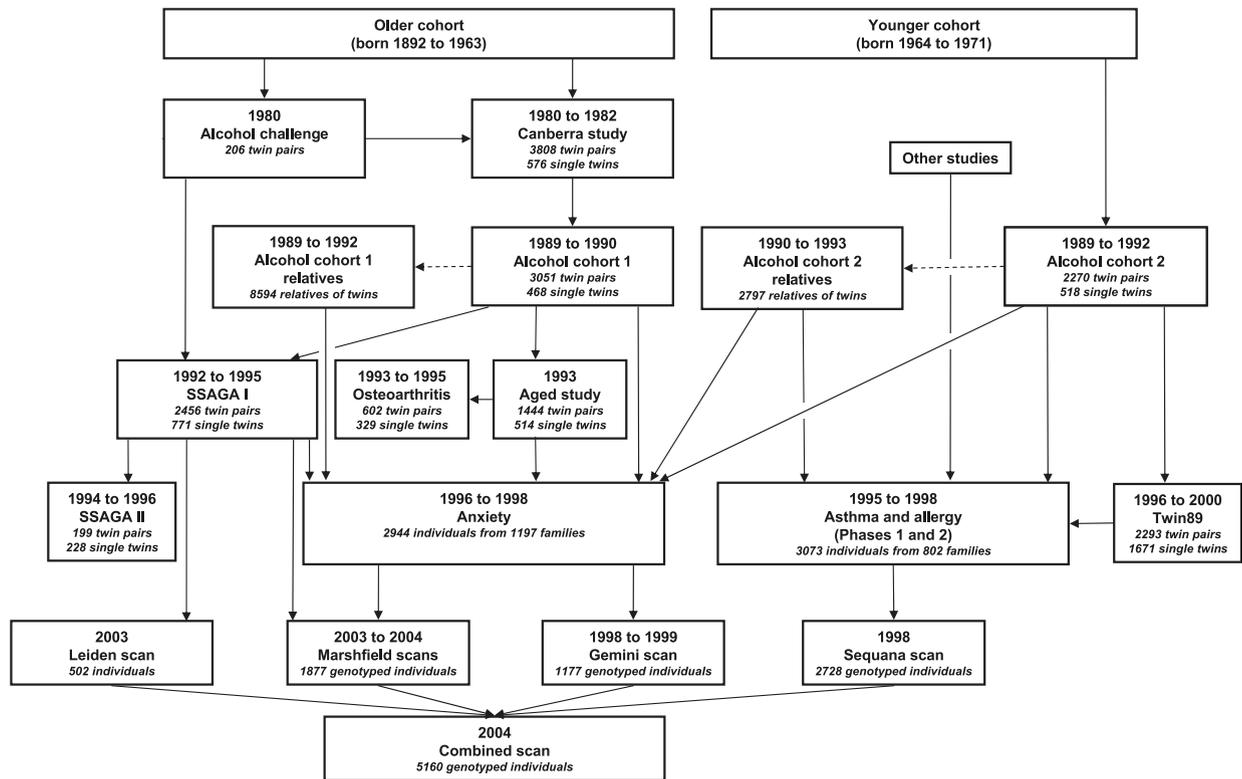Flow diagram illustrating cleaning steps applied to the adult BMI data.

Figure 2

Diagram illustrating recruitment of participants via the various studies. Note that the recruitment process for the Asthma and Allergy study is too detailed to be fully represented here — see Ferreira et al. (in press). Numbers of respondents for each study are given in italics.

summarizes the properties of each individual dataset after inconsistencies between self-reported familial relationships and relationships inferred from genotyping data were resolved (see below).

Gemini

Families were originally ascertained as part of a large study of anxiety and depression including 2451 individuals (Kirk, Birley, et al., 2000). A subsample of 1144 individuals from 387 families was selected for genotyping due to their extreme neuroticism scores. A 10 centiMorgan (cM) genome scan was performed using in-house microsatellite markers at Gemini Genomics, United Kingdom. This dataset was partially cleaned at Gemini, including Mendelian error checking, and allele lengths were converted to allele numbers according to GDB published alleles that are based on the CEPH population.

Leiden

This sample consisted of 502 individuals from 249 families, each containing a dizygotic (DZ) twin pair and no other relatives, with the exception of two families that included two sets of DZ twins. They were ascertained during the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) study (Heath et al., 1997) and later selected for genotyping due to their high cholesterol levels as part of a study of cardiovascular disease (Beekman et al., 2003). A 9 cM

genome scan was completed in five phases at the Leiden University Medical Centre, the Netherlands. First, chromosome 19 was scanned using in-house markers at 8 cM spacing as described previously (Beekman et al., 2003). Second, chromosomes 1, 2, 6, 7, 8, 11, 15, 16 and 17 were scanned at 18 cM spacing on the basis of early power calculations
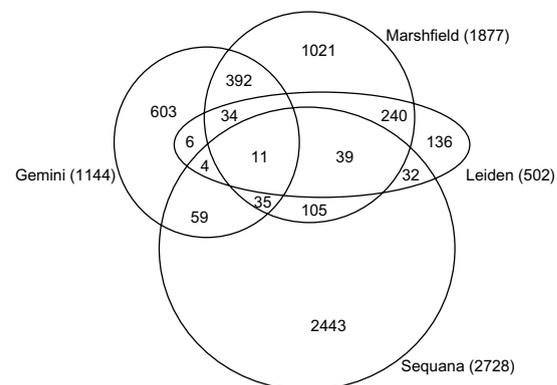

Figure 3

Diagram showing the number of individuals typed per scan (5160 individuals in total), and the overlap between the scans.

The total number of individuals typed in each scan is given in brackets after the scan name.

Table 1

Information for Each of the Genome Scans After Pedigree Errors Were Resolved but Before Genotyping Errors Corrected (Without Marker Cut-Off)

	Individual genotyping datasets				
	Gemini	Leiden	Marshfield	Sequana	Sequana fine-mapping
Individuals	1144	502	1877	558	2170
Families	387	249	687	213	607
All markers	384	435	768	499	120
Autosomal markers	366	416	730	482	120
Total number of genotypes	361,123	158,126	1,303,833	246,966	230,542
Genotypes per marker	987 +/− 129	378 +/− 105	1784 +/− 45	511 +/− 48	1921 +/− 210
Mean +/− *SD* (range)	(223–1124)	(76–493)	(1107–1858)	(257–556)	(36–2115)
Genotypes per individual	316 +/− 41	315 +/− 70	695 +/−68	443 +/− 27	106 +/− 22
Mean +/− *SD* (range)	(25–362)	(4–405)	(5–730)	(240–476)	(2–119)
Marker heterozygosity %	78 +/− 7	78 +/− 9	73+/−8	74 +/− 11	76 +/− 10
Mean +/− *SD* (range)	(43–94)	(4–98)	(27–91)	(18–100)	(37–93)
Intermarker distance cM	9.6 +/− 4.3	8.5 +/−4.5	4.9+/−3.4	6.9 +/− 6.0	7.1 +/− 9.0
Mean +/− *SD* (range)	(1.4–35.3)	(0.01–22.1)	(0.01–43.2)	(0.001–33.7)	(0.001–82)
Information content	0.49 +/− 0.10	0.33 +/− 0.09	0.60+/−0.07	0.40 +/− 0.09	0.72 +/− 0.16
Mean +/− *SD* (range)	(0.30–0.66)	(0.18–0.48)	(0.46–0.71)	(0.21–0.57)	(0.26–0.88)

(Beekman et al., 2001) with markers from Weber screening set 8. Third, additional markers taken from the Human Linkage Set v2.5 MD10 and HD5 were genotyped to increase the marker density. Fourth, the remaining chromosomes were finished with the Human Linkage Set v2.5 MD10. Finally, fine mapping was performed on chromosomes 2 and 19 using markers from the Human Linkage Set v2.5 MD10 and HD5. More details regarding this study can be found in Heijmans et al. (2005). Familial relationships were checked with graphic representation of relationships (GRR; Abecasis et al., 2001), Mendelian errors and unlikely genotypes were flagged and wiped with MERLIN 0.10.1 (Abecasis et al., 2002).

Marshfield

A 5 cM genome scan was performed on 1877 individuals from 687 families at the Mammalian Genotyping Service, Marshfield, United States. These families included twins, parents and siblings ascertained as part of the SSAGA or anxiety studies mentioned above. All individuals were genotyped using markers from the Weber screening sets 13 and 52. Families were selected for genotyping based upon the phenotypic data available for these families regarding cardiovascular risk factors such as lipid levels, adipolipoproteins, exercise levels, smoking, drinking and BMI (Whitfield et al., 2005).

Sequana and Sequana Fine-Mapping

The families included in this dataset were ascertained as part of a large study of the genetics of asthma and atopy (Duffy et al., 1998; Ferreira et al., in press). Families were of arbitrary size and included twins, parents and also for some families, siblings, children,

parents and spouses. Two genotyping events took place. The first event consisted of a 7 cM genome scan of 558 individuals from 213 families that participated in round one. As part of the second event, 120 microsatellite markers were genotyped in 2170 individuals from 607 families across 14 candidate regions: chromosomes 2q24–q35, 3p26–p13, 4q32–q35, 5q31–q32, 7p21–p12, 8p22, 9p24–q21, 11p13–q12, 12q13–12q24, 13q12–q34, 16q21–q24, 17p11–q11, 19p13–q13 and 21q22. In both events, DNA was genotyped at the former Sequana Therapeutics Inc, United States, as described by Hall and Nanthakumar (1997). Familial relationships were verified using GRR. After errors were resolved, alleles were binned and Mendelian segregation inconsistencies identified and wiped with Binning 0.96.0 (Duffy, 2004). Genotypes associated with unlikely recombination events were subsequently flagged and wiped with MERLIN.

Merging of Scans and Cleaning of Combined Data

Pedigree structures for the combined scan were examined using GRR (Abecasis et al., 2001) and RELPAIR version 2.0 (Duren et al., 2003; Epstein et al., 2000) to identify inconsistencies between the genotypic data and self-reported pedigree relationships not identified in the individual scans. Potential pedigree misspecifications, incorrect zygosity labeling of twins, and potential sample mix-ups were identified and investigated. In some instances these problems could be resolved by checking demographic records. In a small number of cases the errors could not be resolved and the problematic individuals or families were removed from further analysis, resulting in the deletion of genotypic infor-

mation for 17 people. Mendelian inconsistencies were identified and cleaned using SIB-PAIR version 0.99.9 (Duffy, 2002).

The combined genome-scan data included 458 unique markers that were typed in two or more scans. One of the difficulties encountered in combining genome scans is ensuring the consistency of the genotypic information for the same markers typed in different genome scans. Instead of collapsing the marker calls for duplicated markers, we included them separately on the genetic map for the combined scan, separated by 0.001cM. These duplicates are counted once toward the total of 458. The consistency of genotype information between these 458 markers was checked using a number of methods. Different genotyping protocols may result in different base pair lengths for the same marker alleles, but this 'shift' in allele length should be consistent across a genome scan sample. Thus by subtracting the marker allele calls of one scan from another, we could assess the relative consistency of two sets of marker genotypes. Unfortunately this strategy could not be used for comparisons with the Gemini data as allele calls were already binned in a manner that did not correspond to allele length. In these cases we assessed marker consistency by the cross-tabulating of allele calls between different scans. Where the results from these assessments suggested that marker data from at least one scan was problematic, we also examined the number of Mendelian errors the marker had produced in each individual scan prior to merging (as calculated by SIB-PAIR), and whether or not the marker was in Hardy–Weinberg equilibrium in each scan (as calculated using PED-STATS; Wigginton & Abecasis, 2005). Genotypic data for 168 of these unique markers were inconsistent between different genome scans. Consequently, the data from one or more of the scans were removed from further analysis.

Unlikely genotypes were identified and omitted from further analyses using MERLIN version 0.10.1 (Abecasis et al., 2002). GENEHUNTER version 2.1 r5 beta (Kruglyak et al., 1996) and MENDEL (Lange et al., 1988) were used to identify and examine potential map errors. Map positions were in Kosambi cM, estimated via locally weighted linear regression (http://www.qimr.edu.au/davidD/) from the NCBI Build 34.3 physical map positions and published Decode and Marshfield genetic map positions (Kong et al., 2004). This map was converted to Haldane cM prior to MERLIN analyses. Where the results suggested inconsistencies between genetic map distances and recombination fractions, the primer sequences for all markers in the region were blasted against the entire human genome sequence (http://www.ensembl.org, NCBI Build 34.3). The genetic map used was then revised to include the updated physical positions of all markers in problematic regions, with new map distances interpolated as above. Using the revised map,

the original genotype data (with inconsistent duplicated markers removed) were cleaned of unlikely genotypes using MERLIN, and map errors were resolved using GENEHUNTER.

To examine the effect of the data cleaning on power to detect linkage we simulated 100 replicates of a phenotype with a 10% QTL effect and a residual autosomal additive genetic effect of 40%. The QTL effect was linked to a marker on chromosome 12 that was located 4 cM upstream of a region in which major map and duplicate problems had been found. We ran MERLIN variance components linkage (at a 2cM grid) for the simulated traits before and after cleaning to assess differences in the power to detect linkage. The per cent of replicates in which a peak with a p value of less than .05 was recorded at the linked region increased from 45% to 52% after cleaning, while the per cent of replicates with a p value of less than .01 increased from 23% to 26%. A similar increase in power was observed with a simulated trait with a QTL effect of 25%. It is possible that the small increase in power may in part reflect the location chosen for the simulation and that a greater increase in power may have been detected in another region or for a trait linked to a marker in which duplicate problems were observed. We conclude that our extensive cleaning procedure has removed errors and produced a modest increase in power for linkage

The cleaned genome scan data includes 1770 autosomal markers, of which 394 are duplicates, leaving a total of 1376 unique markers. The remaining marker overlap between the four genome scans is shown in Figure 4. The mean intermarker distance for all sib-pairs in the sample was 7.1 cM, calculated for each sib-pair and then analyzed across all sib-pairs.
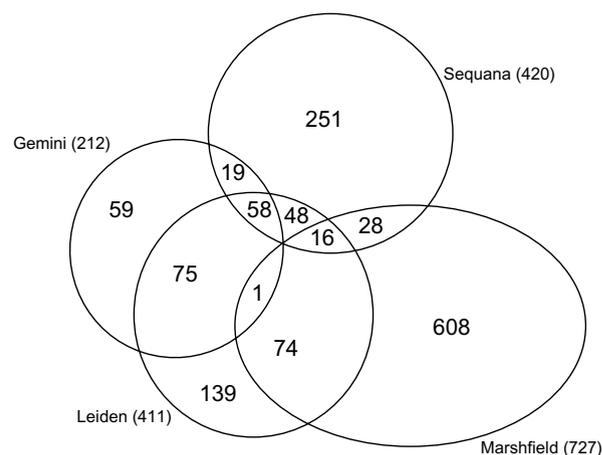

Figure 4

Diagram showing the number of *autosomal* markers typed per scan (1376 unique markers in total), and the overlap between the scans, after cleaning.

The total number of markers typed in each scan in given in brackets after the scan name.

Table 2

Information on Combined Genome Scan Data at Three Different Stages

	Raw	Cleaned	Cleaned and cut off
Individuals	5160	5160	2649
Families	1587	1587	1023
Sib-pairs	2893	2893	1398
All markers	2086	1848	1848
Autosomal markers	1994	1770	1770
Total number of genotypes	2408786	2080944	1800154
Genotyped individuals per marker Mean +/– SD (range)	1157 +/– 670 (76–2659)	1175 +/– 689 (89–2658)	1015 +/– 639 (88–1857)
Genotyped markers per individual Mean +/– SD (range)	448 +/– 356 (5–1853)	419 +/– 355 (5–1714)	680 +/– 261 (198–1647)
Genotyped sib-pairs per marker Mean +/– SD (range)	607 +/– 421 (2–1407)	622 +/– 430 (2–1405)	530 +/– 420 (2–1118)
Genotyped markers per sib-pair Mean +/– SD (range)	418 +/– 332 (3–1725)	381 +/– 320 (2–1544)	672 +/– 220 (91–1544)
Marker heterozygosity % Mean +/– SD (range)	75 +/– 9 (18–98)	75 +/– 9 (18–98)	75 +/– 9 (23–98)
Sib-pair intermarker distance cM Mean +/– SD (range)	7.1 +/– 2.9 (2.7–47.7)	8.1 +/– 4.7 (2.8–49.3)	6.1 +/– 2.9 (2.8–19.2)
Information content Mean +/– SD (range)	0.46 +/– 0.07 (0.33–0.61)	0.41 +/– 0.07 (0.32–0.58)	0.58 +/– 0.05 (0.46–0.67)

Note: Raw = data merged but no genotyping errors corrected; cleaned = genotyping and duplication marker problems resolved; cleaned and cut off = with individuals genotyped for less than 198 markers.

The combined genome scan includes a total of 5160 individuals from 1587 families. Other statistics for this scan, such as marker heterozygosity and information content are detailed in Table 2.

Linkage analyses were performed on individuals with at least 198 markers. This represents 90% of the number of markers in the smallest scan (Gemini, which has 220 markers after cleaning) and is close to the median for that scan (196.5). From this genotypic sample using the 198 marker cut-off, BMI measures were available for 2053 offspring (1649 twins and 404 siblings) from 933 families (114 monozygotic [MZ] and 819 DZ). Following phenotypic cleaning, 65.1% of males and 63.3% of females for whom we had both genotypic and phenotypic data had a clinical measurement. All participants were born between 1910 and 1971 (median year of birth, 1954) and the mean age of female participants was 38.9 years of age (SD 12.7 years) and for males, 37.8 years of age (SD 11.1 years). To assess the reliability of self-reported BMI measurements, we examined the correlation between clinical and self-reported measures in individuals where both types of information were available. In this sample, 1316 individuals (832 females, 484 males) who were chosen based upon having a clinical measurement also had a self-report measure of BMI, with mean error for BMI (measured minus self-reported values) being 2.39 kg/m² for females and 2.07 kg/m² for males. In addition, we calculated the correlation between self-report and measured BMI, which was .82 for females and .72 for males.

Statistical Analysis

Preliminary Analysis

The distribution of BMI was positively skewed, so we transformed the data using the natural logarithm to obtain a normal distribution. Studies have shown that postpregnancy weight retention is a problem for many

women (Gutersohn et al., 2000); therefore, the number of births was tallied until the time of the BMI measurement taken and used as a covariate for women, although this did not factor into the phenotypic cleaning rules. Sex, age at time of measurement, and squared age, Sex × Age, Sex × Age², births, Births × Age and Births × Age² were also tested for their significance in model fitting. Of these variables, sex, age, age² and births showed strong association with BMI, while others could be dropped from the model without a worsening of fit. Outliers exceeding 3 standard deviations from the mean were identified and were excluded from the analysis.

Genetic Analysis

Prior to conducting the modeling analysis, co-twin correlations were computed to estimate the degree of similarity in BMI between members in a pair as well as guiding model selection (ACE, ADE) for biometric analyses. After adjustment for covariate effects, the within-pair correlations by zygosity were: MZ female pairs r_{MZF} = .73; MZ male pairs r_{MZM} = .87; DZ female pairs r_{DZF} = .38; DZ male pairs r_{DZM} = .49; and DZ opposite-sex pairs r_{DZOS} = .38. These within-pair twin correlations showed that DZ same-sex co-twin correlations were higher than half MZ correlations, which suggests that common environmental factors influence BMI in adults, and therefore an ACE model was fitted. Furthermore, assumption testing analyses of the transformed data showed a significant difference in the covariance of BMI between males and females, suggesting the presence of sex differences for this phenotype. Hence, a general nonscalar sex-limitation model was fitted to the data in order to account for different variances of BMI in each sex.

The ACE general nonscalar sex-limited model allows us to address two questions regarding sex differences: firstly, does the same set of genes influence variation in BMI among males and females,

and secondly, are there sex differences in the magnitude of the genetic and environmental variance components? More specifically, this model allows the magnitude of the additive genetic (A), common environmental (C) and unique environmental (E) parameters to differ for males and females (A_M, C_M, E_M and A_F, C_F, E_F respectively) as well as estimating additive genetic influences specific to males (A'_M). In the current study, attempts at parsimony of the sources of variance are guided by Akaike's Information Criterion (AIC): AIC = $\chi^2_{gof} - 2df$ where χ^2_{gof} is the model goodness-of-fit and df is the difference in degrees of freedom between the current model and the preceding model (Akaike, 1987), a lower AIC indicating a better fitting model.

Linkage Analysis

MERLIN was used to calculate multipoint estimates of the probabilities of the sib-pairs sharing 0, 1 or 2 alleles identical by descent (IBD) every 5cM along the genome. For a given marker locus, the estimated proportion of alleles shared IBD is given by = 1/2 p(IBD = 1) + p(IBD = 2) where p is the probability that a given sib-pair shares alleles IBD at a given marker locus.

Through the use of structural equation modeling (SEM) in Mx (Neale, 2002), the proportion of alleles shared IBD was used to estimate another component of variance (Q), which is due to variation at a QTL. The best fitting univariate genetic model was extended to incorporate separate QTL parameters for males (Q_M) and females (Q_F) that were allowed to differ in size (Medland, 2005). To allow the familial covariance to be apportioned into polygenic additive variance and shared environmental variance, 3034 ungenotyped MZ twin pairs were included in the model. A standard linkage submodel in which the QTL variance components for males and females were constrained to be equal was also fitted. Both models were compared to a model in which male and female QTL parameters were set to zero. Models were compared using the likelihood ratio chi-square test.

For the model including separate QTL parameters for males and females, the test statistic is distributed as approximately $1/4\chi^2_0 : 1/2\chi^2_1 : 1/4\chi^2_2$ (Medland, 2005). For the model in which the QTL parameters are constrained equal in males and in females, the distribution of the test statistic is $1/2\chi^2_2 : 1/2\chi^2_0$ (Medland, 2005). To facilitate comparison of linkage results from the two analyses with different distributions, both sets of results are presented as $-\log_{10}p$. Significant linkage was measured by a recommended $-\log_{10}p$ of 4.66, suggestive as $-\log_{10}p$ of 3.13, and replication of an existing finding as a recommended $-\log_{10}p$ of 2, in keeping with Lander and Kruglyak (1995). Due to the amount of time needed to run a single replication of the data (approximately 4 days using Mx), it was impractical to compute empirical $-\log10p$ scores.

Table 3

Tests of Alternative Models of Sources of Variation in BMI After Transformation Using the Natural Logarithm and Adjustment for Covariates (Sex, Age, Age^2 and Births)

Model	V_{Af} (95% CI)	V_{Cf} (95% CI)	V_{Ef} (95% CI)	V_{Pf} (95% CI)	h^2_f (95% CI)	V_{Am} (95% CI)	V_{Cm} (95% CI)	V_{Em} (95% CI)	$V_{A'm}$ (95% CI)	V_{Pm} (95% CI)	h^2_m (95% CI)	$\Delta\chi^2$	Δdf	compared against	p	AIC
1. Saturated	16.63 15.14–17.80	0.03 0.00–0.69	7.13 6.70–7.60	23.79 22.74–24.91	0.70 0.64–0.72	8.60 4.06–11.38	2.12 0.00–5.01	5.13 4.72–5.58	0.00 0.00–0.00	15.85 14.95–16.83	0.54 0.26–0.70	(−2LL = 26486.700 df = 8030)				
2. drop A'_M	16.63 15.35–17.80	0.03 0.00–0.65	7.13 6.70–7.60	23.79 22.74–24.91	0.70 0.66–0.72	8.60 5.66–11.38	2.12 0.00–5.00	5.13 4.72–5.58	—	15.85 14.95–16.83	0.54 0.36–0.70	0.00	1	1	1.00	−2.00
3. Equate V_A	11.71 10.68–12.77	3.99 2.62–5.40	7.44 6.70–7.92	23.13 22.14–24.20	0.51 0.46–0.56	11.71 10.68–12.77	0.00 0.00–0.58	4.98 4.60–5.40	—	16.73 15.78–17.75	0.70 0.66–0.73	28.66	1	2	0.00	26.66
4. Equate V_C	16.66 15.14–17.82	0.00 0.00–0.00	7.14 6.71–7.62	23.80 22.75–24.92	0.70 0.65–0.72	10.73 9.26–11.73	0.00 0.00–0.00	5.11 4.71–5.56	—	15.84 14.95–16.81	0.68 0.59–0.71	2.18	2	2	0.14	0.18
5. Equate V_E	17.06 15.68–18.28	0.15 0.14–1.18	6.39 6.08–6.72	23.61 22.56–24.73	0.72 0.68–0.75	6.87 3.87–10.21	2.99 0.00–5.98	6.39 6.08–6.73	—	16.26 15.37–17.23	0.42 0.24–0.62	37.07	2	2	0.00	35.07
6. Drop C*	**16.66 15.14–17.82**	—	**7.14 6.71–7.61**	**23.80 22.75–24.92**	**0.70 0.65–0.72**	**10.73 9.26–11.73**	—	**5.11 4.71–5.56**	—	**15.84 14.95–16.81**	**0.68 0.59–0.71**	**0.00**	**4**	**1**	**1.00**	**−2.00**

Note: * Best fitting model. Variance components multiplied by 10. Comparisons between models were based on the goodness-of-fit between the model and the data (AIC) as potential components of variation were removed. A indicates additive genetic variance common to both sexes, A' indicates estimates of unique additive genetic effects unique to males, C indicates shared environmental variance estimates, and E represents unique environmental variance estimates. Estimates for females and males are denoted by subscript F and M respectively.

Results

Preliminary Analysis

In order to normalize the data, we transformed them using the natural logarithm which improved skewness (0.49 compared to 1.01) though did not remove it. After the removal of outliers (N = 7 families), data were available for 2036 individuals from 926 families (114 MZ, 458 DZ same-sex pairs [DZSS], 354 DZOS) aged between 18 and 83 years of age (mean age 37.7 years, SD 9.2 years). The mean BMI for males was 25.24 kg/m^2 (N = 760) and mean BMI for females was 24.44 kg/m^2 (N = 1276).

Genetic Analysis

The general nonscalar sex-limitation model was fitted, simultaneously allowing for the effects of the covariates. The standardized variance component results from fitting this model to the data are shown in Table 3. Male-specific additive genetic influences accounted for a small proportion of the total variance and could be dropped from the model without a change in fit. The additive genetic and unique environmental variance for males and females could not be equated (χ_1^2 = 28.66; p < .001 and χ_1^2 = 37.07; p < .001 respectively), but the common environmental variance components could be equated without a worsening of the model fit (χ_1^2 = 2.18; p = .140).

The proportion of variance ascribed to genetic factors (heritability) was .70 in females and .68 in males. The total phenotypic variance under the AE model was larger in females (V_P = 23.80) than males (V_P = 15.84).

Linkage Analysis

As the general nonscalar sex-limitation genetic analyses showed that the variance components of males and females differed, we fitted a general nonscalar sex-limited linkage model to the data that allowed the magnitude of the QTL variance to differ between the sexes (sex-limited) as well as constraining them to be equal (non sex-limited). In addition, we fitted a unique QTL parameter for females (Q'_F) as a higher total phenotypic variance was observed for this sex. When Q'_F was set to zero and compared to the model when Q'_F was free, the difference between the likelihoods of these models did not produce a significant result at any marker and therefore these results are not presented here. The results of the autosomal sex-limited and non sex-limited genome scans are shown in Figure 5.

The highest peak had a $-\log_{10}p$ = 3.02 (near the threshold for suggestive linkage, $-\log_{10}p$ = 3.13) which was observed at approximately 75cM at 12q24 flanked by marker D12S88. The peak on chromosome 12 lies directly over a location which has been associated with noninsulin-dependent diabetes mellitus (NIDDM), a complication of severe obesity. Also identified on Figure 5 are regions known to harbor genes to influence weight regulation, particularly chromosomes 1 (70cM–122cM)

(LEPR), 11 (48cM–90cM) (UCP2 and UCP3), and 12 (47cM–114cM) (GNβ3, ACACB and SCARB1). Table 4 summarizes these findings as well as areas with a $-\log_{10}p$ greater than 1, in particular, regions on chromosomes 7 (159cM–190cM), 8 (0cM–19cM), 14 (59cM–95cM) and 17 (107cM–129cM) wherein a $-\log_{10}p$ greater than 2 (threshold for the replication of an existing finding) were observed that are within the vicinity of regions previously identified by studies on obesity-related phenotypes. For some areas of the human genome, the QTL effects were stronger in one sex, particularly on chromosomes 8 and 20. For example, the locus on chromosome 20 appears to have a stronger effect in males than in females, accounting for 20.5% of the variance in males compared to 0.08% in females.

Discussion

This study aimed to explore sex differences in genetic and environmental contributions to BMI in an adult population, both in the context of the classical twin design and by linkage analysis. In our sample, mean BMI was slightly higher among males than in females, a phenomenon that has also been reported in earlier studies (James et al., 2001; Schousboe et al., 2003). Clinical measures of height and weight were only available for two thirds of this sample, the remaining sample having only self-report measurements available. Because self-reports are biased on self-perception, calculating BMI from self-report measures would result in a lower estimate of overweight adults in the current sample. The correlation between self-report and measured height was high (.91 for females and .90 for males) and similarly, between self-report and measured weight (.85 for females and .80 males). Therefore, the correlation between measured and self-report BMI was high (.82 for females and .72 for males), resulting in BMI estimates with low errors. These results suggest that self-report measures in this sample are reliable, although self-reported weight and height are often under- and overestimated, respectively and therefore should be used with caution.

In all models fitted to the data, we corrected for sex, age (including a quadratic term), and for women, number of births. Significant effects were observed for these covariates, indicating the importance of age-related weight gain in both males and females. The importance of including number of births as a covariate for women also provides further evidence that childbirth may be an environmental trigger affecting genetic expression of BMI in women (Korkeila et al., 1991, 1995; Schousboe et al., 2003).

Genetic Analysis

Overall, results of the genetic analysis lend support to the presence of sex differences in the magnitude of genetic and environmental influences on BMI. Results from both males and females confirm those from other twin studies reporting the importance of genetic
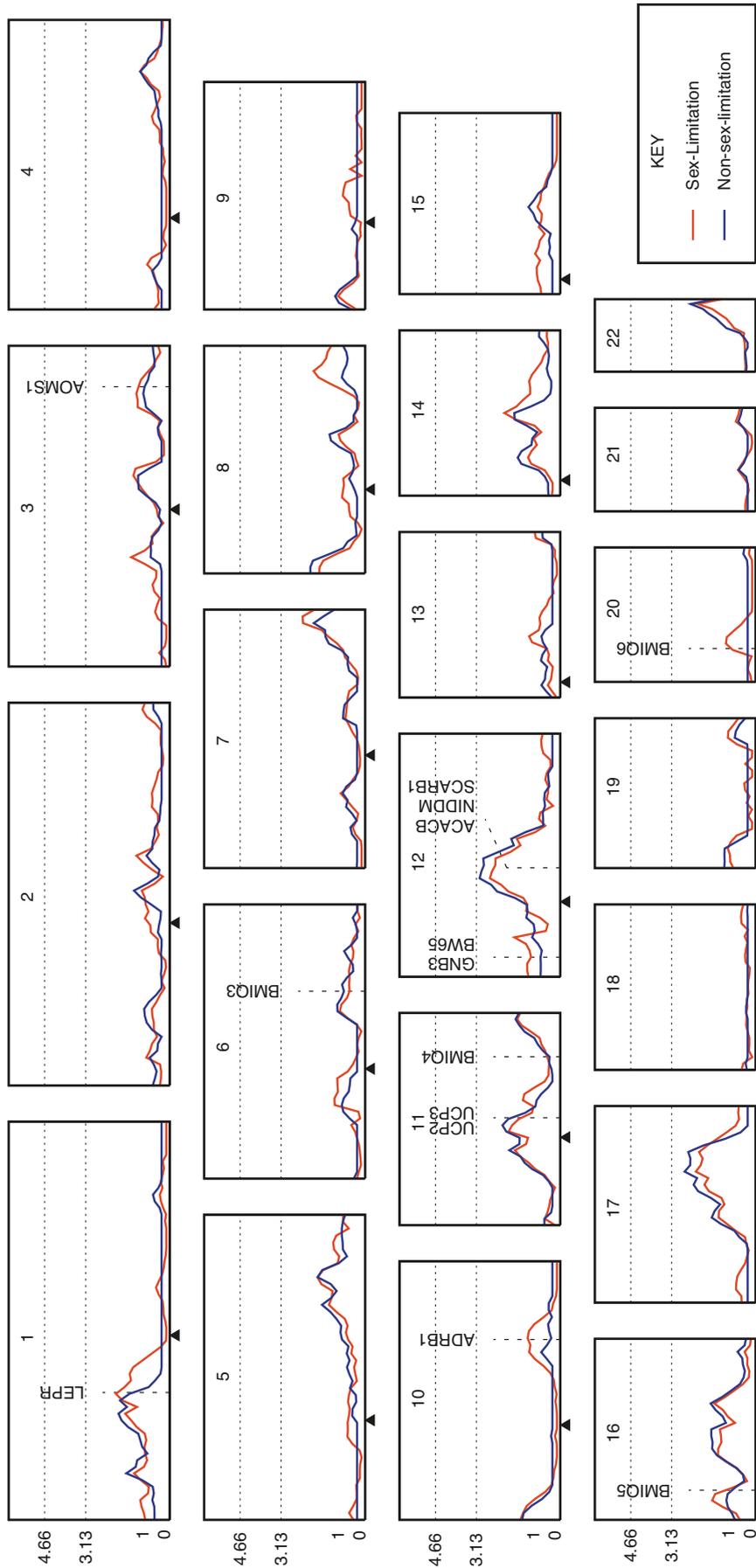
Figure 5

Results of the linkage analysis for natural logarithm transformed adult BMI after adjusting for covariates.

Each panel represents one chromosome and the x-axis is proportional to chromosome length with centromeres indicated by filled triangles. The y-axis shows −log10p values, and lines at 4.66 and 3.13 on the y-axis represents the significant and suggestive values for genome-wide significance. Locations of some candidate genes are shown.

Table 4

Most Interesting Findings Found in the Genome Analysis of Adult BMI

Chromosome	Marker region	Sex-limitation			Non sex-limitation		Phenotypes	Reference
		highest peak $(-\log_{10}p)$	Q'_F	Q'_M	highest peak $(-\log_{10}p)$	Q		
1p31.3	D1S405- D1S3728	2.004	0.526	0.025	1.500	0.231	LEPR — leptin receptor	OMIM 601007
3q27	ATA57D10–D3S2398	1.075	0.353	0.005	0.812	0.138	AOMS1 — Abdominal obesity-metabolic syndrome QTL1	OMIM 605552
7q36	AGAT049 — q terminus	2.333	0.661	0.067	1.555	0.278	Leptin (Older Order Amish) Abdominal total fat	Hsueh et al. (2001) Rice et al. (2002)
8p23	p terminus — ATAA009	1.710	0.334	0.594	2.039	0.433	BMI, paternal effect (in whites)	Gorlova et al. (2003)
8q23	GATA50D10–D8S272	1.916	0.526	0.012	0.801	0.148		
10q24–26	D10S597–D10S670	1.217	0.350	0.000	0.339	0.018	ADRB1 — adrenergic, beta-1-, receptor	Dionne et al. (2002)
11p	CA39_2–D11S1980	1.693	0.499	0.087	1.906	0.287		
11q13	TTA008–D11S987	1.928	0.145	0.520	2.164	0.280	UCP2 — uncoupling protein 2 UCP3 — uncoupling protein 3	OMIM 601693 OMIM 602044
11q24	GATA64D03	0.573	0.223	0.011	0.660	0.101	BMIQ4–BMI QTL 4	Atwood et al. (2002)
12p13	GATA4H03–D12S99	1.609	0.032	0.371	1.670	0.114	GNB3 — guanine nucleotide binding protein (G protein), beta polypeptide 3 BW65 — body weight QTL 65	Gutersohn et al. (2000) Chagnon et al. (2001)
12q24	D12S83–D12S1052	2.623	0.360	0.560	3.015	0.439	NIDDM2 — noninsulin-dependent diabetes mellitus 2 MODY3 — maturity-onset diabetes of the young, type III ACACB — acetyl-CoA carboxylase-beta SCARB1 — scavenger receptor class B, member 1	Mahtani et al. (1996) OMIM 600496 Lewis et al. (2005) Lewis et al. (2005)
14q24	GGAA4A12–D14S258	2.091	0.584	0.038	1.719	0.284	Abdominal subcutaneous fat	Rice et al. (2002)
	D14S74	1.403	0.428	0.001	0.609	0.096	Leptin (Older Order Amish)	Hsueh et al. (2001)
	D14S280–D14S302	1.056	0.317	0.000	0.450	0.049	Leptin (Older Order Amish)	Hsueh et al. (2001)
15q	D15S1507–D15S153	0.849	0.232	0.174	1.191	0.207		
16q21	GATA67G11–D16S421	1.224	0.392	0.111	1.496	0.246	Leptin	Walder et al. (2000)
17q21.32	D17S791–D17S1868	1.164	0.130	0.402	1.325	0.235	Abdominal subcutaneous fat (Quebec Family Study)	Perusse et al. (2001)
17q23.2	AAT245–D17S1290	1.907	0.317	0.490	2.312	0.383	Abdominal subcutaneous fat (Quebec Family Study)	Perusse et al. (2001)
17q24	D17S1291	2.232	0.348	0.548	2.651	0.422		
17q25.2	D17S1301	2.109	0.569	0.158	2.517	0.402	Abdominal subcutaneous fat (Quebec Family Study)	Perusse et al. (2001)
20p11	D20S115–D20S189	1.106	0.002	0.324	0.301	0.000	BMI in 5- to 11-year-old children	Gorlova et al. (2003)
22q13	GATA030–TCTA015	2.150	0.281	0.561	2.425	0.410		

Note: Estimates for the QTL parameter under the sex-limited and constrained model are shown for each region.
Female and male variances are denoted Q'_F and Q'_M, respectively, and the constrained estimate as Q.

and unique environmental influences (Schousboe et al., 2003). The heritabilities of BMI for females and for males were similar (70% and 68% respectively). However, the total phenotypic variance was larger in females (mainly due to the large additive genetic variance) than in males. The source of this extra variance is most likely related to a multitude of biological explanations. Sex differences in the variation attributed to genetic factors are expected due to male–female differences in fat distribution, deposition and accumulation in overall body composition. Leptin (a hormone which acts as a satiety factor and involved in food intake) appears to play a role during pregnancy, suggesting that the interaction between adipose tissue and the reproductive system is modulated in a different way in males and females by androgenic and estrogenic hormones (Casabiell et al., 2001; Hardie et al., 1997).

Linkage Analysis

As the results of our variance components analysis suggested a difference in the magnitude of genetic effects on BMI between males and females, we fitted linkage models that allowed us to assess sex differences in the magnitude of QTL effects. In this sample, only a few of the QTLs identified (although not significant) appeared to have a stronger influence in a particular sex. For example, on chromosome 20, the QTL identified accounted for a much greater proportion of variance in males than in females. On the other hand, on chromosome 8, the QTL identified accounted for a much greater proportion of variance in females. This area located on chromosome 20 has been previously implicated in obesity. Gorlova et al. (2003) located a QTL on 20p11.2 to be involved in the variation of BMI in 5- to 11-year-old children, but did not assess sex differences. This region may thus warrant further investigation in an adult sample.

Using the model in which QTL effects were equated across the sexes, we found suggestive linkage to a region on chromosome 12. Our highest peak was located on chromosome 12q24 which was near the threshold for suggestive linkage. Linkage to this region, between 69cM and 106cM, has been associated with percentage body fat in an African American and Caucasian female population (Lewis et al., 2005). Additionally, there are a number of potential candidate genes for BMI located in this area. Previously identified as likely candidate genes by Lewis et al. (2005) are the acetyl-CoA carboxylase-beta (ACACB) and scavenger receptor type B member 1 (SCARB1) genes located at 12q24.1 and 12q24.31, respectively. The ACACB gene is thought to control fatty acid oxidation in muscle tissue, by means of the ability of malonyl-CoA to inhibit carnitine palmitoyl transferase I, the rate-limiting step in fatty acid uptake and oxidation by mitochondria (Lewis et al., 2005). This gene has been implicated in the development of Type 2 diabetes (Lenhard & Gottschalk, 2002). The SCARB1 gene is a key component in the reverse cholesterol transport pathway where it binds HDL-C with high affinity and is involved in the selective transfer of lipids from it. It is expressed primarily in the liver and non-placental steroidogenic tissues and mediates selective cholesterol uptake. Koumanis et al. (2002) found that the SCARB1 IVS5 C>T polymorphism occurred more frequently in an obese population (BMI > 40) when compared to individuals with a BMI of less than 30, indicating its role in the development of obesity. Our linkage results provide more support for the potential involvement of these genes in obesity-related traits.

A susceptibility gene for NIDDM, a complication of prolonged obesity, is also located in the 12q24 region identified by our linkage analysis. Maturity-onset diabetes of the young, type 3 (MODY3) is a rare, dominant, early-onset (under the age of 25) form of diabetes. This type of MODY is caused by mutation in the hepatocyte nuclear factor-1- alpha gene (HNF1A; Fajans et al., 2001) which is mapped to chromosome 12q24. Mahtani et al. (1996) also identified significant linkage to NIDDM at 12q24 near D12S1349 in a Swedish-speaking population in Finland. However, these Finnish families had an age of onset typical for NIDDM, unlike MODY3, and Mahtani et al. (1996) inferred the existence of a gene NIDDM2, suggesting that NIDDM2 and MODY3 may represent different alleles of the same gene. The clustering of these genes in the same area on chromosome 12 suggests pleiotropic effects with respect to obesity and diabetes.

In addition, our results provide replication of previous linkage findings on chromosome 11. We identified a peak on chromosome 11q13 which was above the threshold for replication of an existing finding ($-\log_{10}p = 2$). Hanson et al. (1998) and Atwood et al. (2002) have previously reported areas on chromosome 11 to be associated with BMI but none of these are located in the same area on the chromosome as the peaks identified in our research. However, uncoupling proteins 2 (UCP2) and 3 (UCP3) are located under the region we observed on chromosome 11. UCP2 is widely expressed in human tissue whereas UCP3 expression seems to be restricted to skeletal muscle, an important site of thermogenesis in humans. There has been much research investigating polymorphisms in these genes that may contribute to obesity through the effects on energy metabolism. For example, female carriers of the UCP3 C-55T polymorphism have a lower risk of obesity (Damcott et al., 2004) and a study of Pima Indian children concluded that UCP2 (or UCP3) contributes to variation in metabolic rate which may affect overall body fat content later in life (Walder et al., 1998).

Limitations and Future Directions

A potential complication of using this data was the large age range. In the current sample, the age range is between 18 and 83 years of age (20% of the individuals in the current sample were aged between 18 and 29 years; 38% between 30 and 39 years; 22% between 40

and 49 years; and 20% between 50 and 83 years of age). In previous studies, age ranges have been narrower, with younger cohorts being between 20 and 29 years of age and older cohorts being between 30 and 39 years of age (Neale & Cardon, 1992; Schousboe et al., 2003). Between these two cohorts, large differences have been reported, with nonadditive genetic effects identified in the younger cohort (Neale & Cardon, 1992) as well as sex-specific genetic effects influencing adult BMI in the younger cohort (Schousboe et al., 2003). Therefore, in these analyses, we may be observing a gene-by-age effect on BMI. Future studies addressing these issues through the incorporation of age in a genotype-by-environment moderator model are planned (Purcell & Sham, 2002).

In the current study, we have confirmed the importance of genetic factors in the variation of BMI. The results of our linkage analyses presented here suggest the presence of a number of chromosomal regions that may harbor genes involved in obesity. Some genes that are known to play a role in the development of obesity are located in these regions. Although we did not find significant evidence for linkage to these regions, gene–gene and/or gene–environment interaction may potentially hide the true effects of these genes. Obesity is a multifactorial disease, and only rare forms of obesity appear to be due to monogenic effects. In the case of polygenic obesity, susceptibility genes, taken individually, will only have a slight effect on weight, and it is the cumulative contribution of these genes that influences obesity, especially when exposed to certain environmental factors (Clement, 2005).

Identification of significant linkage results for this sample may have been hampered by the inconsistent genotypic data that was available. The genotypic data used in this analysis was a composite of genome scan data from four sources. While combining the data from these different sources provided more information for linkage analysis, a negative consequence of this was that not all individuals were typed in all four genome scans. Thus individuals were not typed for a consistent number of markers, even within families. Nonetheless, we believe that the multipoint algorithm used in the estimation of IBD probabilities minimizes the impact of this, as shown by the observed average information content of the cleaned genome scan (0.58).

Additionally, our use of a sex-limitation linkage model preserved our sample size. Previous studies examining sex differences in QTL effects have typically assessed linkage separately in male and female sibling pairs. However, in the case of an unselected sample such as this one this approach would result in discarding approximately half of the sib-pairs. Thus, the sex-limitation model adopted here provided a more powerful test of sex differences in the QTL component in the context of the current sample. In the future, we plan to incorporate more genotypic and phenotypic information into the current sample which is likely to improve the linkage results.

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