Meta-Analysis of Genome-wide Linkage Studies in BMI and Obesity

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Abstract

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Objective: The objective was to provide an overall assessment of genetic linkage data of BMI and BMI-defined obesity using a nonparametric genome scan meta-analysis.

Research Methods and Procedures: We identified 37 published studies containing data on over 31,000 individuals from more than >10,000 families and obtained genome-wide logarithm of the odds (LOD) scores, non-parametric linkage (NPL) scores, or maximum likelihood scores (MLS). BMI was analyzed in a pooled set of all studies, as a subgroup of 10 studies that used BMI-defined obesity, and

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for subgroups ascertained through type 2 diabetes, hypertension, or subjects of European ancestry.

Results: Bins at chromosome 13q13.2- q33.1, 12q23-q24.3 achieved suggestive evidence of linkage to BMI in the pooled analysis and samples ascertained for hypertension. Nominal evidence of linkage to these regions and suggestive evidence for 11q13.3-22.3 were also observed for BMI-defined obesity. The FTO obesity gene locus at 16q12.2 also showed nominal evidence for linkage. However, overall distribution of summed rank p values <0.05 is not different from that expected by chance. The strongest evidence was obtained in the families ascertained for hypertension at 9q31.1-qter and 12p11.21-q23 ($p < 0.01$).

Conclusion: Despite having substantial statistical power, we did not unequivocally implicate specific loci for BMI or obesity. This may be because genes influencing adiposity are of very small effect, with substantial genetic heterogeneity and variable dependence on environmental factors. However, the observation that the FTO gene maps to one of the highest ranking bins for obesity is interesting and, while not a validation of this approach, indicates that other potential loci identified in this study should be investigated further.

Key words: diabetes, hypertension, genetics, meta-analysis, adiposity

Introduction

The fundamental causes of the rise in obesity in developed and increasingly in developing countries are unknown but are thought to relate to a change in energy balance resulting from the reduced energy expenditure associated with increasingly sedentary lifestyles and energy dense diets (1,2). The cultural effects on body weight observed after people migrate to Western countries and the rapid changes in the prevalence of obesity (occurring over a single generation) point to a significant role for environmental causes, such as socioeconomic and cultural factors (3). Genetic factors are also very important: substantial evidence for a genetic component in body weight regulation and adiposity comes from family, twin, and adoption studies (4,5). Heritability estimates reported from family studies are in the range of 25% to 40%, and for twin studies in the range of 50% to 80% (6). The complex interaction between inherited susceptibility to and high-risk environments for developing obesity mean that approaches to obesity management should consider all of these causes (7).

Both linkage and association analysis have been used to attempt to find susceptibility genes for BMI and obesity. Although the linkage approach in particular has been successful in identifying the 6 known single-gene forms of obesity (8), these are very rare in the general population, accounting for <1% of cases.

There have been more than 30 genome-wide linkage scans and several hundred candidate gene association studies of BMI, and approximately 10 genome-wide linkage scans of obesity, with a variety of loci and genes implicated in the disorder (9). However, no susceptibility genes for common, complex forms of obesity or body weight regulation have been unequivocally identified by these methods. Individual genome-wide scans for linkage have identified a number of genetic loci that reach significance criteria, and some of these have appeared in more than one study, such as those on chromosomes 2p, 6q, 20p (8). Thus, although evidence has emerged for specific loci linked to BMI and obesity (9), none has been consistently implicated in the majority of genome scan projects.

There are many possible explanations for the observed inconsistency of results of linkage scans using BMI as a phenotype. Some of these are methodological, and others relate to disease etiology, such as locus heterogeneity (i.e., more than one separate genetic susceptibility loci), the involvement of different biological pathways, genetic and environmental differences between populations, variability in ascertainment criteria, and so on. If there is extensive heterogeneity, each locus is likely to have a small population-wide effect on susceptibility to obesity or on BMI, which is difficult to detect consistently without very large samples.

Methodological factors influencing consistency of linkage studies may include differences in statistical strategies, the reporting of spurious loci more than once (given the large number of studies), or differences in sample size and statistical power (10). Many studies of BMI and obesity have used small sample sizes, although some large studies [e.g., National Heart, Lung, and Blood Institute Family Heart Study (11)] do exist. Strategies that might overcome these problems include very large population-based linkage studies, which would increase statistical power, studies that take into account environmental factors, or genome-wide association strategies, all of which should be able to detect genes of smaller effect.

Another possibility is meta-analysis of linkage data, where the gain in statistical power might overcome the problems of locus heterogeneity and clarify the role of individual loci and assist the mapping effort. Consequently, we applied the rank-based genome scan meta-analysis (GSMA)[1] method (12) to data from the 37 complete genome scans of BMI, 10 of which are specifically for obesity. The GSMA is a widely used nonparametric method for combining results from published genome scans and can be applied to a combination of studies with different markers, family structures, and analysis methods. In previous studies,

[1] Nonstandard abbreviations: GSMA, genome scan meta-analysis; LOD, logarithm of the odds; NPL, non-parametric linkage; MLS, maximum likelihood scores; FUSION, Finland-United States Investigation of NIDDM Genetics.

the method has identified significant evidence for linkage that was not evident in the individual studies (13–15). If loci with small effect, consistent across studies, exist for BMI or obesity, then this approach would have power to identify them.

Research Methods and Procedures

GSMA Method

The genome was divided into 120 bins of approximately 30 cM. For each study, results were assigned to bins, and the strongest evidence for linkage within each bin was identified [e.g., the highest logarithm of odds (LOD)-, Z- or non-parametric linkage (NPL)-score, or the minimum p value]. For most studies, evidence for linkage for each bin [LOD scores, NPL scores, maximum likelihood scores (MLS), or p values] was taken directly from graphs of genome-wide linkage results as published in the original papers. Each graph was imported into a drawing program (CorelDraw) and overlaid with a grid dividing each chromosome equally into the required number of bins. These were then ranked in order of evidence for linkage by measuring the peak height within each bin using a graphical ruler from within the drawing program. For the remaining studies (16–31), linkage scores were obtained by request from the study authors, who sent us the full genome-scan results. A control comparison between ranks estimated this way, and those from linkage results data for the same study showed correlation of >0.9. For studies (11,17,20, 23,24,27,32–34) where results included marker names, bins were determined using predefined markers (see www.kcl.ac.uk/depsta/memoge/gsma/bin_definitions.html for listing of bin boundary markers). For all other studies, each chromosome was divided into equal width bins. Bins are referred to by chromosome, so "bin 1.4" indicates the fourth bin on chromosome 1.

The data for the GSMA consist of a maximum linkage statistic for each study and each bin. Within each study, the bins are ranked, with the bin achieving maximum evidence for linkage assigned rank 120. For each bin, the ranks are then summed across studies, and this summed rank forms the test statistic for that bin. Under the null hypothesis of no linkage, the summed rank is the sum of random ranks chosen from (1, 2, >.120) with replacement. Bins with high summed ranks therefore show evidence for linkage.

The summed rank is tested for significance by simulation, permuting the bin location of ranks within each study. For each bin, two tests of significance are performed. The summed rank p value is equal to the proportion of simulated bins with summed rank greater or equal to the observed summed rank. For the ordered rank p value, the "place" of the bin's result is noted, and compared with the summed rank of bins with the same "place" in each simulated GSMA. So, for the observed highest summed rank, the ordered rank p value is calculated from the summed ranks of the bins attaining highest summed rank in each GSMA simulation. Similarly, the second highest summed rank is compared with the second highest values in each complete simulation. Simulation studies have shown that bins with both significant summed rank and ordered rank p values have a higher probability of being true linkage findings (35).

We used three different thresholds for significance: genome-wide significance using a Bonferroni correction for testing 120 bins ($p = 0.05/120 = 0.00042$), suggestive significance expected to occur once in each GSMA study ($p = 1/120 = 0.0083$), and nominal significance ($p = 0.05$).

The GSMA analysis was carried using the GSMA software (36), available from http://www.kcl.ac.uk/depsta/memoge/gsma/download.html, with p values based on 10,000 simulations. Analyses were performed unweighted and weighted for study size. In the weighted analysis, ranks were multiplied by the study weight before summation across studies (see below for choice of weighting function).

Ascertainment of Studies

The inclusion criteria for studies in this meta-analysis were whole genome scans of BMI, analyzed both as quantitative and categorical trait. The latter was defined by dichotomization of BMI at a cut-off point. Our underlying hypothesis is that the same quantitative trait loci will influence variation to both BMI and BMI-defined obesity, which we think is a reasonable hypothesis given the scarcity of single-gene forms of obesity and the fact that BMI-defined obesity is very common (BMI ≥30 in more than >25% of the U.S. population; http://www.cdc.gov/nccdphp/dnpa/obesity/trend/maps/index.htm). Studies were identified in two main ways. Whole genome scans were identified from the obesity gene database (37) or were selected by using searches for combinations of "genome scan," "genome search," "linkage," "BMI," "body mass index," and "obesity" using Medline, searching between April and July 2004. Some additional studies were identified on an ad hoc basis, from the cited references in other papers (30) cited by a previous meta-analysis (38), or using internet Web-based search engines (39). In addition, we included data from our own unpublished linkage study of BMI [using the Genetic and Environmental Nature of Emotional States in Siblings (GENESIS) sample described by Nash et al. (17)] and one based on the Finland-United States Investigation of NIDDM Genetics (FUSION) sample described by Silander et al. (31). These studies were included because we were aware of the data from our own study, and data from the Silander et al., study was offered to us by the investigators as it was a direct, independent extension of the initial FUSION study by Watanabe et al. (30). The inclusion of these studies based on our awareness of them should not introduce bias into our analysis, as their inclusion is independent of the results obtained in any specific genome region. All included studies were identified by July 2004, except Watanabe et al.

(30), which was included in July 2005. Studies that investigated linkage in only one region were excluded because the GSMA method is based on ranking linkage information across the entire genome.

To ensure that individual studies were included only once, certain decisions were made about which papers to include, because some studies are represented in the literature multiple times (e.g., as an individual study and as part of a meta-analysis). The data from the Framingham Heart Study gave a particular problem (40) because several different analyses of genome-wide linkage for BMI have been carried out on the same data (41–49). A subset of the Framingham data, however, has also been included as part of a further study (11) and so this further study, but none of the additional or original analyses, was included. The results of Lee et al. (50) were a subset of a second analysis (19) and were excluded. Genome scans of obesity traits other than BMI-defined obesity, such as leptin or other anthropometric measures, were excluded in an attempt to perform a meta-analysis on a relatively homogenous phenotype. Studies whose analyses were primarily assessing parent of origin effects on BMI, e.g., (51), were also excluded.

Despite these strict inclusion criteria, 37 genome-wide linkage analyses of BMI were eligible for inclusion (11,16–34,39,52–57). These include the two unpublished genome-wide linkage analyses of BMI: one based on the sample included in Nash et al. (17) and one based on the sample of Silander et al. (31). The study from Wu et al. (53) included 8 scans, which were analyzed separately. 34 of 37 included studies have authors listed for this meta-analysis as part of the GSMA-BMI consortium. Table 1 gives details of the ascertainment criteria and the sampling populations for the studies included in this analysis, and a summary of the analysis methods used in the original studies. We were not able to obtain data from one published study (58).

With regards to the studies with obesity as ascertainment criteria, 10 published genome-wide linkage studies were identified, 7 of which used BMI as a categorical trait to define obesity (i.e., obesity = BMI $>$30 kg/m2) and 3 of which used BMI as a quantitative trait. In 2 of the 10 studies, obesity was investigated in childhood/adolescence, and in these the family ascertainment criteria were a proband with BMI $>$95th centile and a sibling with BMI $>$90th or $>$95th centile, respectively (29,52). The ascertainment criteria for the proband in the adult studies were extreme BMI ($\geq$35 or BMI $\geq$40), and for the siblings, a BMI cut-off point of between $\geq$27 and $\geq$40. Table 1 gives further details of the obesity studies.

Some studies performed different analyses and/or considered different BMI phenotypes. Stone et al. (20) looked at three phenotypes (BMI, BMI in females, and BMI in males) and performed four different parametric and nonparametric analyses. We used the results of the analysis across both sexes and considered for each bin the maximum LOD score obtained from the parametric linkage analysis across dominant, codominant, and recessive models. Li et al. (19) analyzed 4 different thresholds for obesity status; we used the linkage results from only one of these in the meta-analysis, the cut-off point at BMI $\geq$30. The majority of studies did not examine linkage by sex and did not include the X chromosome resulting in too few studies to attain sufficient statistical power for their analysis. Consequently, the GSMA analysis was not performed separately for males and females, nor was the X chromosome included. Likewise, the majority of studies did not stratify patients by age but used a range of age groups within and between families, and only two analyzed children only. Thus, we did not perform stratification by age.

GSMA Analyses

To identify potential common susceptibility loci for both BMI and obesity, a combined analysis including all of the 37 genome-wide analyses was performed. For studies where BMI was analyzed as quantitative trait, weighting by the number of genotyped individuals was used. This was scaled to give an average weight of 1. Weights ranged from 0.30 to 4.6. For obesity studies where BMI was analyzed as a categorical trait, a weighting function of the number of affected individuals was used, giving a maximum weight of 2.4 and a minimum weight of 0.5. Separate weighting schemes were chosen to reflect the approximate informativeness of each individual: for the obesity studies where BMI was analyzed as a categorical trait, each affected individual will contribute more information than a genotyped individual in the studies where BMI was analyzed as a continuous trait, whose phenotype is not selected for the tail of the distribution. For the pooled analysis, adjusted study weights were used, so analysis consisted of 28 "continuous trait" studies with a mean weight of 1, and 7 "categorical trait" studies, with a mean weight of 1.

Two studies, Li et al. (19) and Stone et al. (20), reported data with BMI analyzed both as a continuous and a categorical trait, and both analyses were used in the meta-analysis. In the Li et al. study (19), results from the categorical and quantitative analysis are quite discordant, so they were included in the meta-analysis as if they had been independent studies. However, the two Stone analyses show a strong concordance in results, each set of results was therefore weighted by one half the original weighting factors calculated for the Stone et al. (20) datasets. Genome-wide linkage for BMI was calculated in FUSION 1 study (30) separately for subjects with type 2 diabetes and their unaffected spouses/children; and although the same families were used, the results are considered independently as study subjects were only included in one of the two analyses.

Two major sources of heterogeneity in the linkage studies are the phenotype of ascertainment and population. BMI is an easily measured phenotype, and in some studies the

Table 1. Characteristics of whole genome studies of BMI, subdivided by ascertainment criteria

Study name or site	First author, year (reference)	Population	Ascertainment	Phenotype and adjustments*	Number of families	Number of subjects	Analysis Method	Program
Obesity								
Analyzed as categorical trait								
Utah	Stone (2002) (20)	Utah	3 members: BMI ≥40	Severe obesity BMI ≥40	64	640	NPL analysis	MCLINK
Pennsylvania	Li (2004) (19)	European American	Prob: BMI ≥40; sib: BMI ≥30	Obesity (BMI ≥30)	203	609	Nonparametric	GH
France	Hager (1998) (26)	French	Prob: BMI ≥40; sib: BMI ≥27	Obesity (BMI >27)	158	424	Nonparametric	MMS
France	Meyre (2004) (29)	French	2 sibs: BMI >95%; before age 8	Obesity (BMI >95%)	115	329	Nonparametric	GH
France	Bell (2004) (28)	French	2 sibs: BMI ≥35	Obesity (BMI ≥35)	109	240	Nonparametric	GH
Germany	Saar (2003) (52)	Germany	Prob: BMI ≥95%; sib: BMI ≥90%; mean age: 13.6 ± 2.8	Obesity	89	191	Multipoint LOD score analysis	MLBGH
Finland	Ohman (2000) (27)	Finnish	Prob: BMI ≥40 sib: BMI ≥32	Obesity (BMI ≥32)	87	188	Nonparametric	MMS
Analyzed as quantitative trait								
TOPS	Kissebah (2000) (34)	White American	Obesity	BMI	507	2209	VC	SOLAR
Utah	Stone (2002) (20)	Utah	Extreme BMI	BMI	64	1687	MCMC	MCLINK
Pennsylvania	Li (2004) (19)	European American	Prob: BMI ≥40; sib: BMI ≥30	BMI	260	1297	Regression	MERLIN REGRESS
Type 2 diabetes								
FUSION 1	Watanabe (2000) (30)	Finland	Type 2 diabetes	LOG BMI	580	1175	VC	GH2β
FUSION 1	Watanabe (2000) (30)	Finland	Unaffected spouses/ children prob: type 2 diabetes	LOG BMI	210	715	VC	GH2β
FUSION 2	Silander (2004) (31)	Finland	Type 2 diabetes	LOG BMI#	242	580	VC	GH
Arizona	Hanson (1998) (22)	Pima Indians	Type 2 diabetes	LOG BMI	264	966	VC	VC
Amish Family Diabetes Study	Hsueh (2001) (18)	Amish	Type 2 diabetes	BMI	28	672	VC	SOLAR
Breda Study Cohort	van Tilburg (2003) (23)	Dutch	Type 2 diabetes	LOG BMI	178	562	VC	GH2
SAFDS	Arya (2004) (21)	Mexican American	Type 2 diabetes	BMI, T2 days, leptin	27	403	VC	SOLAR
Japan	Iwasaki (2003) (39)	Japan	Type 2 diabetes	LOG BMI	164	368	VC	GH2
Hypertension								
HyperGEN	Wu (2002) (53)	Black American	Essential hypertension	BMI	800*	1256	VC	GH2
HyperGEN	Wu (2002) (53)	White American	Essential hypertension	BMI	1055*	1138	VC	GH2
SAPPHIRE	Wu (2002) (53)	Japan, China	Blood pressure	BMI	-	1070	VC	GH2

Table 1. Continued

Study name or site	First author, year (reference)	Population	Ascertainment	Phenotype and adjustments*	Number of families	Number of subjects	Analysis Method	Program
GENOA	Wu (2002) (53)	Mexican American	Essential hypertension	BMI	468*	788	VC	GH2
GENOA	Wu (2002) (53)	White American	Essential hypertension	BMI	924*	753	VC	GH2
GenNet	Wu (2002) (53)	Black American	Hypertension	BMI	297*	621	VC	GH2
GENOA	Wu (2002) (53)	Black American	Essential hypertension	BMI	893*	617	VC	GH2
GenNet	Wu (2002) (53)	White American	Hypertension	BMI	384*	606	VC	GH2
Nigeria	Adeyemo (2003) (32)	Nigeria, Yoruba	Hypertension	BMI	182	769	VC	SOLAR
Obesity-correlated traits								
NHLBI Family Heart Study	Feitosa (2002) (11)	White American	High risk of CHD	BMI	718	4211	VC	SEGPATH
Cleveland Family Study	Palmer (2003) (54)	White	Obstructive sleep apnoea	BMI, alcohol, smoking, airway surgery	66	349	VC	SOLAR
Cleveland Family Study	Palmer (2004) (55)	African American	Obstructive sleep apnoea	BMI, OSA	59	277	VC	SOLAR
Other								
Rochester Family Heart Study	Turner (2004) (24)	non-Hispanic white	Population-based	BMI	279	1848	VC	EMVC
Bogalusa Heart Study	Chen (2004) (25)	White American	Population-based	BMI	342	782	VC	SOLAR
GENESIS	Nash (2004)* (17)	UK-white	Anxiety/depression index	LOG BMI#	283	708	Regression	MR
Nebraska	Deng (2002) (33)	European American	Low bone-mineral density	BMI,BMD	53	630	VC	SOLAR
Finnish Twin Cohort	Perola (2001) (16)	Finland	Combined analysis of 5 substudies (hypertension, obesity, migraine, osteoarthritis, hyperlipidaemia)	1/BMI	247	614	VC	SOLAR
HERITAGE	Chagnon (2001) (56)	White American	Population-based	BMI	99	552	Regression	MMS
Netherlands	Heijmans (2004) (57)	Dutch	Anxious depression	BMI	192	525	VC	MERLIN

Every study adjusted the BMI phenotype by age and sex, with the exception of the TOPS and the Bogalusa Heart Study. Abbreviations for the programs used in each original study are: EMVC, expectation-maximization variance component; MCLINK, multipoint component linkage; Merlin, multipoint engine for rapid likelihood inference, and MERLIN-REGRESS, Merlin family regression method; MLBGH, maximum likelihood binomial method; MMS, Mapmaker/Sibs; GH, GH2beta, and GH2, Genehunter, Genehunter v2.0 beta version, Genehunter v2.0; SEGPATH, segregation and path analysis; SOLAR, sequential oligogenic linkage analysis routines; VC, variance-components approach. Please refer to the original articles for more details.

T2, type 2 diabetes; CHD, coronary heart disease.

Contain BMI linkage data, which is unpublished and was obtained directly from the investigators.

* Reported number of sib pairs, rather than the number of families.

families were originally ascertained for the presence of a different phenotype (e.g., obesity, type 2 diabetes, hypertension). One assumption of the meta-analysis is that the genes that regulate BMI are the same in hypertensive and diabetic populations, and in the different racial and ethnic groups included. However, restricting the combined analyses to studies with similar ascertainment criteria or to subjects from a similar ethnic background may increase the power to detect linkage to particular regions. Subgroup analyses of the families ascertained through obesity, diabetes, and hypertension were therefore performed, with 8 studies included in the diabetes subgroup (18,22, 23,30,31,39,41) and 9 studies in the hypertension subgroup (32,53). In addition, 27 studies with subjects of European ancestry were also analyzed separately (11,16–18,20,23–25,30,31,33,34,46,53–57). Other population groups had too few studies available for a separate analysis.

Results

Results for the meta-analyses of all of the BMI studies (pooled analysis) and of the obesity studies, weighted by study size are shown in Figure 1A and B, which shows genome-wide results (i.e., with a Bonferroni correction for testing 120 bins) with thresholds for significance (1%, 5%, 10%). Results for the subgroup analyses (pooled, obesity, diabetes, hypertension, and subjects with European ancestry) are shown in Figure 2, which lists bins achieving a nominally significant summed rank (p value >0.05) for the weighted (pW) or unweighted analyses (pU). No bin achieved significant evidence for linkage at a genome-wide level ($p < 0.00042$), and few bins achieved suggestive evidence for linkage ($p < 0.0083$). For most analyses, there was strong consistency across the bins identified in the weighted and unweighted analysis. We focus on the weighted analysis in reporting results.

Bins 13.2 or 13.3 reached suggestive evidence for linkage in the pooled analysis (bin 13.2 $pW < 0.009$; bin 13.3 $pW = 0.008$), and nominal significance in families ascertained through obesity (bin 13.3 $pU = 0.019$), with European ancestry (bin 13.2 $pW = 0.016$; bin 13.3 $pW = 0.011$), and in families ascertained for hypertension (bin 13.3 $pW = 0.029$). Significant results in adjacent bins are a common finding in GSMA studies, and are due to the correlation of ranks in adjacent bins, particularly when multipoint analysis methods have been used in the original studies.

Several other regions were implicated by pooled or ascertainment-specific analyses. Bin 12.5 showed a suggestive evidence for linkage in the pooled analysis ($pU = 0.004$) and a nominal significance in the families ascertained through obesity or hypertension. The FTO obesity gene locus at 16q12.2 (59) showed nominal evidence for linkage in the pooled analysis of obesity in bin 16.3. Bins 2.2, 9.5, 11.3, 11.4, 11.5, 17.2, and 17.3 also achieved a summed rank p value ≤ 0.05 in two of the five different analyses. Summed rank p values ≤ 0.01 were obtained not only in the pooled analysis (bins 13.2, 13.3, 12.5), but also in the obesity analysis at bin 11.3 ($pU = 0.007$), for the families ascertained through diabetes at bins 17.3 ($pW = 0.007$) and 4.3 ($pU < 0.001$), for the families ascertained through hypertension at bins 12.3, 9.5, and 12.4 ($pW = 0.004$, $pW = 0.007$, and $pW = 0.008$, respectively) and for the European ancestry group at bin 13.3.

The GSMA uses multiple-testing of 120 bins, so about 6 bins would be expected to attain $p < 0.05$ in each analysis. This is consistent with the number of significant bins in the BMI (pooled analysis), and in the obesity status, European ancestry, and diabetic subgroup analyses. No significant ordered rank p values were observed in these analyses. However, in the hypertensive subgroup (9 studies), stronger evidence for linkage was seen, with 3 bins obtaining a p value <0.01 and six further bins attaining a summed rank p value $pW < 0.05$. In the hypertension analysis, bins 12.5 and 13.3, which arose across different analyses, reached also ordered rank p values < 0.05, increasing the evidence of the existence of susceptibility genes in these regions.

Discussion

We found several regions of the genome that provided suggestive or nominal evidence of linkage to BMI. Bins at chromosome 13q13.2-q33.1 (bins 13.2, 13.3) and 12q23-q24.3 (bin 12.5) achieved a suggestive evidence of linkage for BMI in both the pooled weighted or unweighted analysis, and nominal evidence in the unweighted analysis of the obesity studies. In this latter analysis, suggestive evidence of linkage was observed in the region 11p12-q13.3 (bin 11.3).

However, the results from the GSMA of 37 genome-wide scans on BMI and of 10 specific genome screens on obesity do not provide strong evidence for any particular region being consistently implicated in the genetic contribution to either trait. This is reflected by the fact that, in both the pooled and the obesity analyses, the distribution of summed rank p values <0.05 is not different from that expected by chance, under the hypothesis of no linkage, with ordered rank p values >0.05 for each bin. Moreover, our results do not seem to highlight any common region implicated in both traits. In contrast, a previous GSMA meta-analysis of linkage scans for BMI as a continuous trait was based on data from only five studies (16,18,32,33,58) from 13 studies initially targeted for inclusion. We included data only from those studies for which the authors provided data, despite the fact that this is not necessary where genome-wide data (for example, in the form of figures) is included in the publication. The data from the five studies included 2814 individuals from 505 families (38), and using the same analysis methods, showed strong evidence of linkage to chromosome 8p. The five studies included were heteroge-

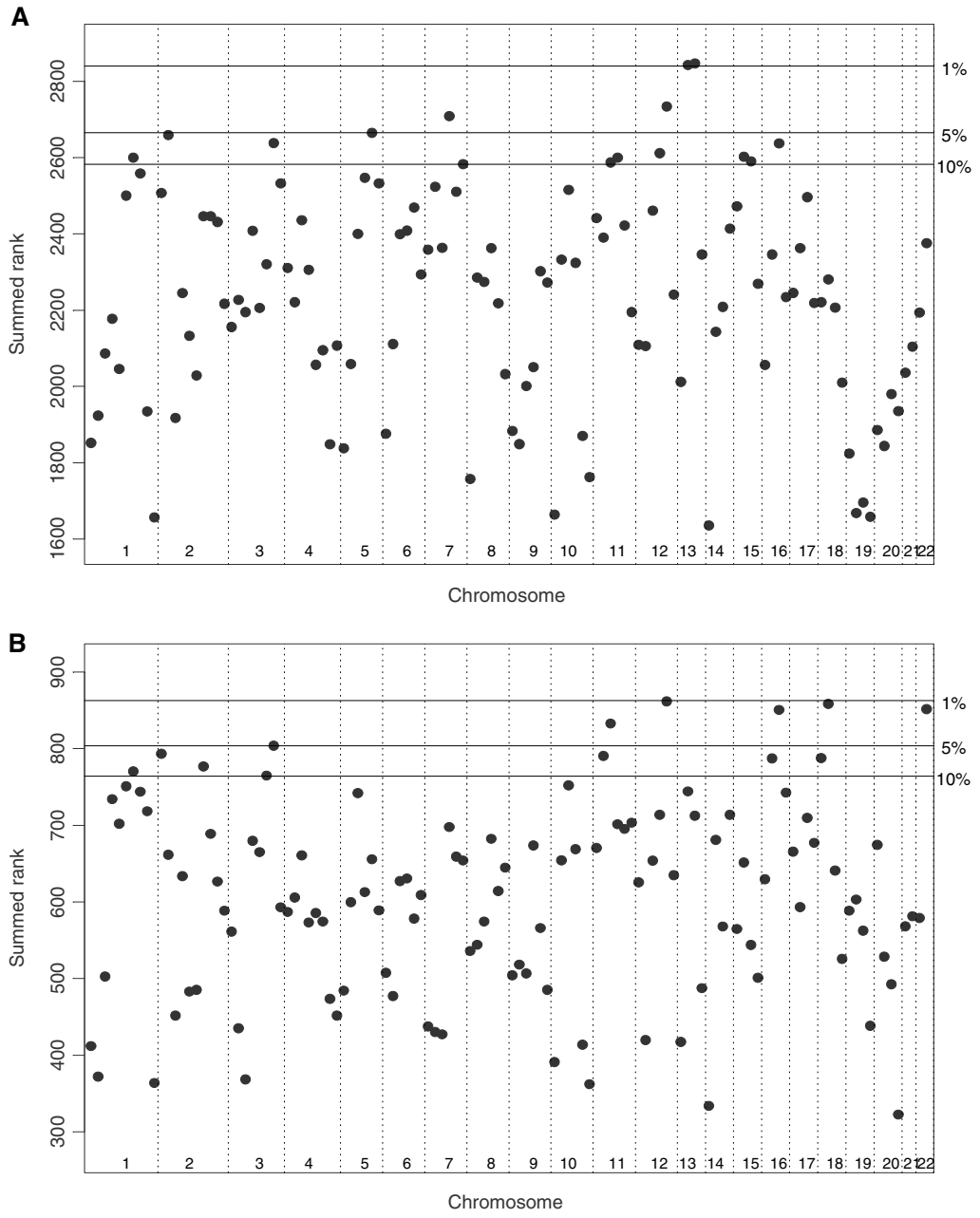

Figure 1: Results of the BMI (pooled) and obesity meta-analysis, showing summed rank for each bin (corrected for genome-wide significance) and confidence limits (10%, 5%, 1%).

neous with respect to both ethnicity and ascertainment, which were respectively hypertension, osteoporosis diabetes, random house-to-house recruitment, and finally obesity, familial dyslipidemia, migraine, osteoarthritis, and hypertension. Thus, the fact that Johnson et al. (38) obtained a positive result whereas we did not is unlikely to result from reduced heterogeneity in their sample.

The results presented here are therefore somewhat surprising, given that this meta-analysis represents a very large number of linkage studies, encompassing data from more than 10,000 families and over 31,000 individuals (including four of the five studies considered previously) and for two traits that are substantially heritable. It is not compatible with common major loci governing BMI and obesity in the general population but points toward the existence of many genes of small effect.

The most likely explanation for our failure to find strongly positive loci is not lack of statistical power, but the

Chr, bin (cM interval)	BMI in Pooled Analysis (37 studies)		BMI on Obesity (10 studies)		BMI on Diabetes (8 studies)		BMI on Hypertension (9 studies)		BMI in studies with subjects of European ancestry (27 studies)	
	W	U	W	U	W	U	W	U	W	U
2. 2 (25.5-48.0)										
2. 8 (177.5-206.7)										
3. 4 (88.6 – 117.8)										
4. 3 (51.6- 79.0)						0.01				
5. 5 (131.5-164.2)										
5. 6 (164.2-197.5)										
7. 1 (0-29.3)										
7. 4 (91.7-122.5)										
7. 6 (148.1-182.0)										
8. 5 (110.2-137.9)										
9. 3 (53.6- 84.9)										
9. 5 (109.9-136.5)							0.007			
9. 6 (136.5-169.0)										
11. 1 (0-21.5)										
11. 2 (21.5-47.0)										
11. 3 (47.0-72.8)				0.007						
11. 4 (72.8-99.0)										
11. 5 (99.0-123.0)										
11. 6 (123.0-147.8)										
12. 3 (53.3-82.1)							0.004	0.01		
12. 4 (82.1-109.5)							0.008			
12. 5 (109.5-139.6)		0.004								
12. 6 (139.6-170.6)										
13. 2 (26.9-58.5)	0.008									
13. 3 (58.5-85.4)	0.009								0.01	
15. 1 (0-25.9)										
16. 2 (32.1-67.6)										
16. 3 (67.6-100.4)										
17. 2 (25.1-63.6)										
17. 3 (63.6-94.0)					0.007					
18. 2 (24.1-62.9)										
22. 2 (33.8-62.3)										

SR p value ≤0.05 SR & OR p value ≤0.05 SR p value ≤0.01

Figure 2: Bins achieving significant evidence for linkage by summed rank p value for weighted (W) and unweighted (U) analysis of all groups. SR, summed rank; OR, ordered rank. The cM distances shown in column 1 correspond to the Marshfield genetic map.

presence of substantial locus heterogeneity (involving several biological systems affecting appetite, behavior, activity levels, metabolism, as well as genetic variation between ethnic groups and according to ascertainment criteria), epistasis, and age-dependent effects. Obesity in rodents is clearly highly heterogeneous, where studies of mice indicate the presence of overlapping combinations of main and epistatic effects at adjacent ages, which have opposite effects on early and late growth. Body composition traits in mice seem to be influenced by an interacting network of multiple quantitative trait loci, which are shared by the body composition and body weight traits, suggesting that pleiotropy plays an important role in growth and obesity (60).

The failure to find loci with genome-wide significance could be partly related to the characteristics of BMI as a phenotype. BMI is the most easily measured proxy for obesity but 1) is a composite of fat and nonfat mass (the latter including bone mass), which are not likely to be regulated in the same way, and 2) may not always represent body fat consistently since the relationship between BMI and fat-mass is not always linear and often shows subgroup differences. Although high BMI values may principally be because of adiposity, in individuals nearer the mean high BMI may be more influenced by other features of body composition such as bone and muscle mass. This may be a particular problem for population-based studies where the majority of individuals are not obese and lie close to the mean. Despite this, it is worth reiterating that BMI is a highly reproducible and heritable phenotype, and the results we observe cannot be a result of flaws in the phenotype

alone. Nevertheless, it is likely that more direct measures of adiposity would improve the consistency of linkage analysis, and it would be interesting to see what a meta-analysis of a more specific measure of body fat (e.g., percent body fat, total abdominal fat, or abdominal visceral fat) would show. However, at present, there are too few linkage studies of these phenotypes (9) to make a meta-analysis viable, in our opinion.

The GSMA method itself has several limitations, and these are discussed in detail elsewhere. The method should have high power to detect genes that can be detected in a linkage analysis (35), although it is difficult to assess the power of this specific study. GSMA might fail to detect linkage to loci where genetic heterogeneity is present, either across studies (because of population differences) or within studies (only a subset of families linked). Tests for heterogeneity in the GSMA are available (61) but have very low power (62). Similarly, the GSMA would miss common genes with a low sibling recurrence risk that would be easily detectable in large association studies, such as the insulin-induced gene 2 (INSIG2) gene, which was recently identified as a probable susceptibility gene for obesity by genome-wide association (63). Since the risk allele has an odds ratio of only 1.2, its effect would be very difficult to detect by linkage, and indeed INSIG2 maps to 2q14.2, which was not identified as a locus for BMI or obesity in this study or in other individual linkage studies of obesity.

Variability of marker density within scans could also have an effect on the GSMA. Because of this, positive findings in the GMSA analysis may be conservative but negative results are less informative; no method can exclude linkage in any chromosomal region for a complex disorder, and GSMA data should not be interpreted in this way.

However, despite our lack of formal evidence for BMI and obesity loci from the meta-analysis, the highest ranking bins have been identified as candidates from other studies. This cannot be construed as formal support for the GSMA results, as BMI and obesity are related to many common traits and diseases, and coincidence of loci could easily occur by chance, but are worth noting as they may assist with mapping, candidate gene analysis, or functional studies.

Bins 13.2 and 13.3, corresponding to bands 13q13.2-q33.1, are the most significant results we observed both in the pooled analysis and in the subgroup with subjects of European ancestry, and achieved a significant ordered rank p values for linkage in the families ascertained through hypertension. One candidate gene from this region is the 5-hydroxy-tryptamine receptor 2A gene polymorphism (5-HTR2A, 46 cM), that resides on 13q14-q21 near to bin 13.2. 5-HTR2A has been associated with dietary energy and alcohol intake in obese people (64), and also with anorexia nervosa (65). Serotonin is a key mediator in the control of satiety mechanisms. Serotonin reduces food intake and is probably involved in weight regulation (66).

Bin 2.2 (2p25.1-p23.2) has already shown linkage with several obesity-related phenotypes in addition to BMI (67), including skinfold thickness (68), leptin (26,69), and adiponectin levels (70). This region contains the gene encoding pro-opiomelanocortin, a locus previously linked to leptin levels and fat mass in Mexican-American, French, and black-American cohorts and shown to be mutated in obese humans. This region also contains the apolipoprotein B gene (2p24.2), which has been associated with BMI and percentage body fat (37).

The most interesting results of the analysis are probably those obtained in the analysis focusing on the families ascertained through hypertension, which provide strong evidence of linkage. The significant ordered rank p values for this analysis show that the clustering of significant p values is more likely to reflect true BMI susceptibility loci in these regions (35). That this subgroup provides the strongest evidence for linkage is perhaps surprising, as the subgroup is quite heterogeneous, with some researchers having excluded all diabetics in order to focus on "essential" hypertension. On the other hand, many hypertensive subjects also have a metabolic syndrome. In this analysis, we included 9 studies, 8 of which had already been analyzed together (53), and so we would expect a concordance in the results. However, Wu et al. (53) identified a region on chromosome 3q using an identity-by-descent sharing analysis, which we did not find. This region showed a strong signal in only one of the 8 included studies. The different meta-analysis methods used may therefore explain these discordant results. Wu et al. (53) used both the IBD sharing method and the Fisher method for meta-analysis, which retain the magnitude of significance of the original studies, while GSMA is a rank based statistic and a single region with a strong linkage signal is less likely to be identified unless it is replicated across studies. In contrast, the region 12q14–15, identified in our analysis, was also highlighted in this previous meta-analysis, although only in the analysis using the Fisher method, nonetheless indicating that this may well be a region of true linkage.

The genes mapping to these loci could either be genes solely relating to BMI, which have nothing to do with hypertension, genes that have a pleiotropic effect on both phenotypes, or genes that are primarily hypertension genes but influence BMI in this population only. A hypertension candidate gene is also already known to be located in the region 12q14–15, arginine vasopressin receptor 1A. In the other regions that we identified here, cyclooxygenase 1 and dopamine beta-hydroxylase are found in 9q32-q33.3 and 9q34, respectively, corresponding to bin 9.5; angiotensinase C/Prolyl-CPY is found in 11q14 (bin 11.4) and endothelin

receptor type B is found in 13q22 (bin 13.3.). No doubt, candidate genes for BMI could also be identified from these regions.

Finally, a comment on the the recent discovery of association between type 2 diabetes and obesity and the FTO gene on chromosome 16 (59). This is one of the most convincing genetic findings in obesity, having been replicated in large samples sizes both within and independently of the original study (71,72). FTO maps to 16q12.2, which is within bin 16.3, one of the highest ranking bins for obesity in the present study (Figure 1B). This indicates that the other putative loci identified by this study, which are orphan loci in the sense that they have no unequivocally identified underlying gene, should be investigated further.

In summary, results from our meta-analysis, the largest GSMA study ever performed, failed to find strongly positive loci for BMI from linkage studies published to date. However, it does contribute to the body of evidence supporting the involvement of some loci in the complex polygenic regulation of BMI. Our findings indicate that BMI is most likely a highly heterogeneous trait, and as a general phenotype for linkage and association analysis is inferior to direct measures of adiposity or body composition, especially if consistent ascertainment criteria are not used.

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