

ORIGINAL ARTICLE

Multicenter dizygotic twin cohort study confirms two linkage susceptibility loci for body mass index at 3q29 and 7q36 and identifies three further potential novel loci

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Objective: To identify common loci and potential genetic variants affecting body mass index (BMI, kg m⁻²) in study populations originating from Europe.

Design: We combined genome-wide linkage scans of six cohorts from Australia, Denmark, Finland, the Netherlands, Sweden and the United Kingdom with an ~10-cM microsatellite marker map. Variance components linkage analysis was carried out with age, sex and country of origin as covariates.

Subjects: The GenomEUtwin consortium consists of twin cohorts from eight countries (Australia, Denmark, the Netherlands, Finland, Italy, Norway, Sweden and the United Kingdom) with a total data collection of more than 500 000 monozygotic and dizygotic (DZ) twin pairs. Variance due to early-life events and the environment is reduced within twin pairs, which makes DZ pairs highly valuable for linkage studies of complex traits. This study totaled 4401 European-originated twin families (10 535 individuals) from six countries (Australia, Denmark, the Netherlands, Finland, Sweden and the United Kingdom).

Results: We found suggestive evidence for a quantitative trait locus on 3q29 and 7q36 in the combined sample of DZ twins (multipoint logarithm of odds score (MLOD) 2.6 and 2.4, respectively). Two individual cohorts showed strong evidence independently for three additional loci: 16q23 (MLOD = 3.7) and 2p24 (MLOD = 3.4) in the Dutch cohort and 20q13 (MLOD = 3.2) in the Finnish cohort.

Conclusion: Linkage analysis of the combined data in this large twin cohort study provided evidence for suggestive linkage to BMI. In addition, two cohorts independently provided significant evidence of linkage to three new loci. The results of our study suggest a smaller environmental variance between DZ twins than full siblings, with a corresponding increase in heritability for BMI as well as an increase in linkage signal in well-replicated regions. The results are consistent with the possibility of locus

heterogeneity for some genomic regions, and indicate a lack of major common quantitative trait locus variants affecting BMI in European populations.

International Journal of Obesity advance online publication, 1 September 2009; doi:10.1038/ijo.2009.168

Keywords: genetic linkage; body mass index; dizygotic twins; quantitative trait locus; heritability

Introduction

Obesity (Online Mendelian Inheritance in Man (OMIM): 601665)-related traits have been a target of numerous studies in recent decades. This vast amount of information can be reviewed on the obesity gene map website (<http://obesitygene.pbr.c.edu/>). Extremes in body mass index (BMI) distribution, that is, both excesses and very low BMI, present serious health problems. A low BMI, usually an indication of protein-energy malnutrition or the effects of wasting or a disease process, is a significant predictor of mortality.¹ On the other hand, high BMI and obesity increases the risk of coronary heart disease, certain types of cancer, hypertension, osteoarthritis and type 2 diabetes mellitus. Several monogenic forms of obesity have been identified which are exemplified by mutations in the leptin and melanocortin receptors. Moreover, several syndromes have been shown to manifest obesity as one of the symptoms, such as the Prader–Willi syndrome (OMIM: 176270), the Bardet–Biedl syndrome (OMIM: 209900), Cohen's syndrome (OMIM: 216550) and the Macrosomia, obesity, macrocephaly and ocular abnormalities syndrome (OMIM: 157980). These monogenic and syndromic forms of obesity are relatively rare and only explain a small fraction of variability in the trait. The first successful genome-wide association study (GWAS) by Frayling *et al.*² showed that relatively common variants of the *FTO* gene affect BMI in the general population. Since then, four other GWASs have shown a number of regions; however, they only account for <1% of the variance in BMI.^{3–6} In recent years, a number of studies have published linkage results with obesity. The obesity gene map database shows that there is an abundance of obesity-linked loci and every chromosome has been linked to obesity, except chromosome Y. Bell *et al.*⁷ conclude in their review paper that seven of these quantitative trait loci have been subsequently replicated (2p21–p23, 3q27, 4q31–q32, 7q31–q32, 10p11–p12, 11q14–q24 and 20q11–q13). For a more

extensive review of the genetics of obesity, see a paper by Bell *et al.*⁷ and a book edited by Clement and Sørensen.⁸ To date, most of the loci found in GWASs have been central nervous system related, suggesting that they are behavioral genes. It is possible that linkage studies will identify different loci than will GWASs. Linkage studies are more powerful to find rare variants with high impact, whereas it cannot detect the common low-impact variants that have been identified by GWASs. Our study aimed at finding loci contributing to variability in BMI in a large cohort of European-originated twins, using variance components linkage analysis. The samples were genotyped from population-based registers and were unselected for BMI or any other phenotype.

Materials and methods

The data sets and methods are described in detail by Perola *et al.*⁹ In brief, genome-wide microsatellite scan data from the following six twin cohorts were available for analysis: The Australian Twin Registry,¹⁰ the Danish Twin Registry,¹¹ the Finnish Twin Cohort,¹² the Netherlands Twin Register,¹³ the Swedish Twin Registry¹⁴ and the TwinsUK Adult Twin Registry.¹⁵ Although these are all twin studies, some of them have recruited additional family members included in this study. Data sets are summarized in Table 1. The combined sample set comprised 10 928 individuals from 4401 families with genotype and phenotype information. The skewness and kurtosis of BMI in the total sample were 1.14 and 2.69, respectively, and therefore a log base10 transformation was used. After normalization, the skewness and kurtosis of logBMI were 0.47 and 0.82, respectively. logBMI was used in all analyses. Sex, age and country of origin correlate significantly with BMI and they were used as covariates in the combined sex analysis. Outliers ($n = 393$), determined by

Table 1 Demographics of sample sets

	Males						Females						No. of families	Average family size	Average map density
	Mean BMI	s.d.	Min	Max	Mean age	n	Mean BMI	s.d.	Min	Max	Mean age	n			
All	25.34	3.44	13.84	53.26	50.15	3667	24.7	4.48	13.64	51.64	48.91	6868	4401	2.4	
Australia	25.38	3.74	14.64	53.26	44.51	1214	24.69	4.64	13.85	48.27	44.44	1876	1287	2.4	7.7 cM
Denmark	25.22	3.31	13.84	36.56	53.69	247	23.51	3.70	14.52	37.07	60.96	377	315	2.0	9.6 cM
Finland	25.49	3.70	16.47	46.00	52.07	518	24.72	5.22	13.64	45.70	60.43	339	387	2.2	9.3 cM
The Netherlands	25.21	3.26	14.87	39.64	45.12	1160	24.46	4.13	14.68	47.48	43.55	1535	757	3.6	9.2 cM
Sweden	25.47	2.72	19.57	35.80	74.62	528	25.17	3.68	16.53	41.88	74.99	525	544	2.0	4.2 cM
United Kingdom	NA	NA	NA	NA	NA	NA	25.08	4.68	15.21	51.64	47.26	2216	1111	2.0	4.7 cM

Abbreviations: BMI, body mass index; NA, not available.

Table 2 Number of informative pairs with both phenotype and genotype information

Relative pair	Pairs
Sib pairs	5837
Half-sibs	27
Cousins	24
Parent-child	3078
Grandparent-grandchild	14
Avuncular	119

values differing by more than 3 s.d. from the population mean, were excluded from the analyses. Marker maps from all of the cohorts were combined using Cartographer program (<http://apps.bioinfo.helsinki.fi/cartographer/>).¹⁶ The program GRR (<http://www.sph.umich.edu/csg/abecasis/GRR/>) was used to test for the validity of twin zygosity and other familial relationships.¹⁷ Genotypes were checked for Mendelian inconsistencies using the PedCheck program (http://watson.hgen.pitt.edu/register/soft_doc.html).¹⁸ The non-Mendelian inconsistencies (2249 of the 5 882 017 genotypes of 6919 loci were excluded) were evaluated with Merlin's¹⁹ genotyping-error option and removed by option pedwipe.¹⁹ The analyses were performed with AUTOGSCAN²⁰ program (<http://www.helsinki.fi/~tsjuntun/autogscan/>) which uses Merlin.¹⁹ We performed variance components analysis for logBMI in the 10 535 individuals. There were a total of 3356 dizygotic (DZ) twin pairs (706 male pairs, 2040 female pairs and 610 opposite-sex pairs). The heritability estimates were calculated in Merlin along with the linkage analyses. The larger proportion of females was because of the UK sample, which was a large female-specific cohort. The linkage analysis was also carried out conditional on sex to examine whether there was a sex-specific contribution to the linkage signal at a given locus. The number of informative pairs with both genotype and phenotype data is summarized in Table 2. The analyses were carried out per country in both extended families (where available) and DZ twins only. We then combined samples across countries and analyzed the combined DZ twin sample and extended families sample, which included all the available individuals.

Results

The covariate-adjusted heritability of BMI was 54% in the extended families sample and 73% in the DZ twin sample. Sex-stratified analyses were carried out in the extended family sample and in the DZ sample, but not in the country-specific samples. Figures 1 and 2 show all of the linkage results per chromosome in the extended family sample, DZ twins-only sample and sex-stratified analyses. The DZ twin data provided evidence for more loci than did the extended family sample. The DZ linkage loci of multipoint logarithm of odds score (MLOD) > 1 are summarized in Table 3 with corresponding MLOD scores from both sample sets. The country-specific linkage peaks of MLOD > 2 are summarized in the Table 4.

Discussion

Body mass index has been an extensively studied trait because of the health effects of obesity. The need for more effective clinical strategies has become evident with the increasing prevalence of obesity in recent years. This highlights the importance of finding variants responsible for differences between individuals in how they respond to factors resulting in an imbalance between energy expenditure and energy intake. Yet, the causes of the increase in obesity are more complex than suggested by the energy imbalance equation. Thus, Keith *et al.*²¹ have shown other putative contributors to the increase in obesity prevalence. They found at least 10 factors which correlated with the increased prevalence of obesity in the United States during the recent decades, for example, increased sleep deprivation, reduction in variability in ambient temperature, decreased smoking, pharmaceutical iatrogenesis (weight gain due to medication) and increasing gravida age. However, the causal role of these temporally correlated factors needs to be established. Hypothetically, genes correlated with obesity may be genes acting on these putative risk factors rather than directly on obesity-related metabolic factors. Within populations, numerous family and twin studies have documented a consistent major role of genetic effects on BMI.^{22,23} The linkage and candidate gene studies have been summarized by the human obesity gene map.²⁴

In this study, we aimed at finding common major loci affecting BMI in European populations. The DZ twins in this study originate from six twin cohorts of European origin. The aims of this multicenter analysis were to replicate previous findings and to use the greater statistical power to identify novel loci. DZ twins are of the same age, have shared the same prenatal and family rearing environment and school experiences more closely than had full siblings from families. As BMI changes with age, and the genetic determinants of weight change are largely uncorrelated with BMI level in adulthood, matching on age is an important advantage of the current data set.^{25,26} As the biggest individual cohort comprised 2216 individuals, power was substantially increased by combining the cohorts which totaled 10 535 individuals. On the other hand, the combined sample would be unable to find population-specific loci.

We showed suggestive linkage in two regions, namely 3q29 and 7q36 (MLOD = 2.6 and MLOD = 2.4, respectively) that were extensively replicated in other studies, thus providing further evidence that these regions harbor genes that affect BMI.

A summary of earlier relevant findings in relation to the current results is shown in Table 5. The linkage to chromosome 3q29 region is exemplified by findings from Kissebah *et al.*²⁸ with numerous metabolic syndrome component traits, including BMI, waist, hip, insulin, insulin:glucose in Caucasian families (MLOD = 2.4–3.5, 2209 individuals from 507 families). Luke *et al.*²⁹ showed linkage with BMI in an African-American population

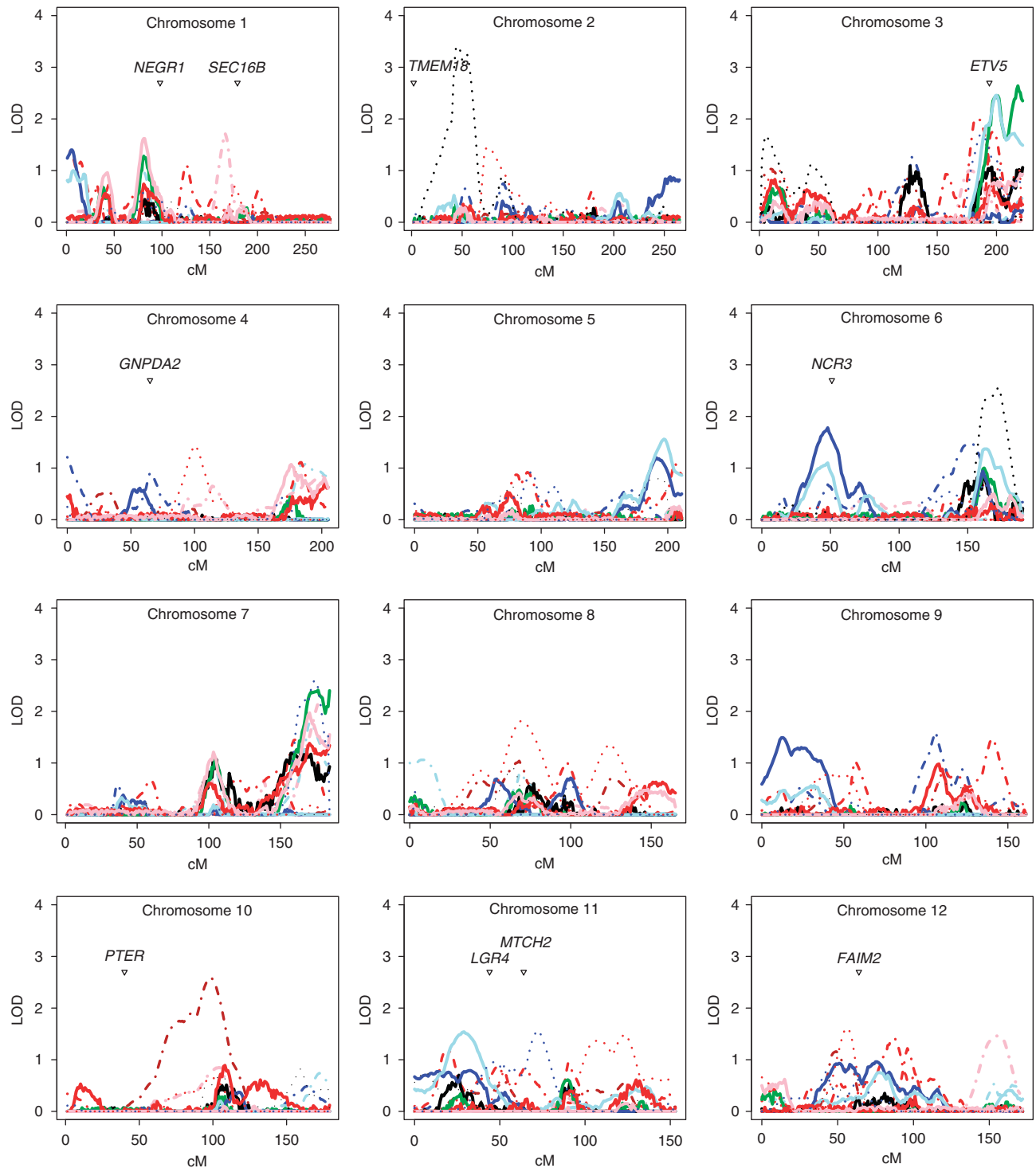


Figure 1 The multipoint variance components linkage results for chromosomes 1–12 and published genome-wide association loci.

(MLOD = 4.3, 1163 individuals from 329 families), and Wu *et al.*³² showed linkage with BMI in a combined sample of European, African and Mexican Americans (MLOD = 3.4, in 4021 sib pairs). The key finding of the chromosome 7q36

region include the following: the study by Feitosa *et al.*,³⁴ who have shown linkage to BMI in an European–American sample (MLOD = 4.9, 3407 individuals from 536 families), and the study by Sammalisto *et al.*,³³ who reported linkage to

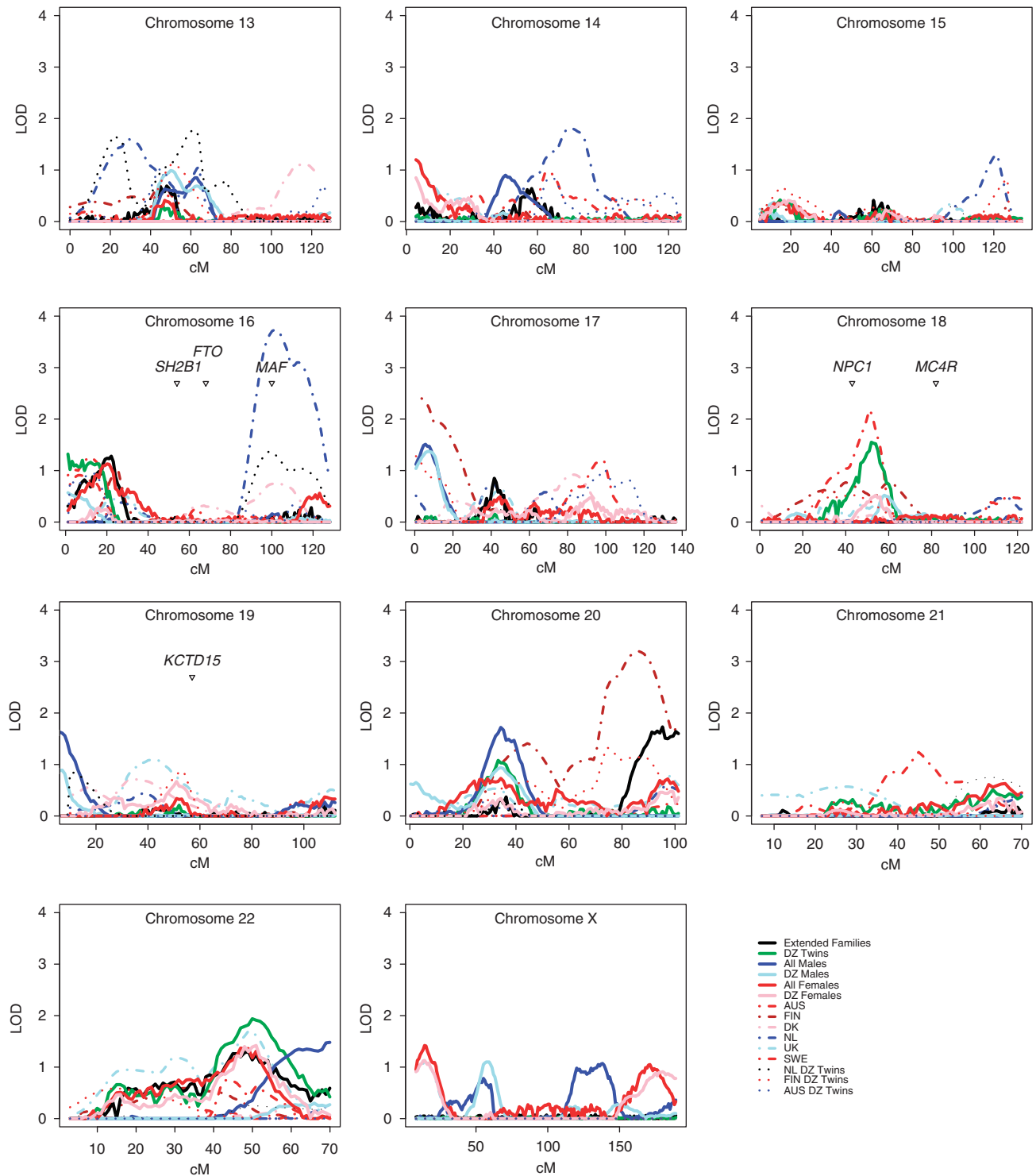


Figure 2 The multipoint variance components linkage results for chromosomes 13–X and published genome-wide association loci.

BMI in a combined African-American and European-American sample (female-specific analysis MLOD = 2.9, of 5788 females from 3032 families). Interestingly, the linkage signal in chromosome 7q36 area of our study is female

driven which is consistent with the study by Sammalisto *et al.*, suggesting a female-specific effect for this locus. There was no sex-specific effect in the chromosome 3q29 linkage signal. There are some interesting candidate genes right

under the best linkage peaks of the combined sample. For example, in the 3q29 region, there is *APOD* (OMIM: 107740), which has not yet been associated with human obesity but has a role in fat metabolism. The linkage peak in 7q36 is near the leptin gene (7q27), but more interestingly under the linkage peak resides the *INSIG1* (OMIM: 602055) gene. It has

Table 3 Linkage peaks with MLOD > 1 in either DZ twins or in extended families

Position	DZ twins	Extended families
1p32.2	1.3	0.3
3q27.3	2.5	0.7
3q29	2.6	0.9
7q36.3	2.4	1.0
16p13.2	0.4	1.3
16p13.3	1.3	0.3
18q12.1	1.6	0.0
20p12.2	1.1	0.3
20q13.32	0.1	1.7
22q13.2	1.9	1.1

Abbreviations: DZ, dizygotic; MLOD, multipoint logarithm of odds score.

Table 4 The cohort-specific linkage results of MLOD > 2 both in DZ twins and extended families

Position	MLOD	Sample set
2p24.1	3.4	Dutch DZ sample
3q26.32	2	Australian extended families
6q26	2.56	Dutch DZ sample
7q36.2	2.6	Australian DZ sample
7q36.3	2.4	Danish DZ sample
10q22.3	2.6	Finnish extended families
16q23.2	3.73	Dutch extended families
17p13.3	2.28	Finnish extended families
18q12.1	2.2	Swedish DZ sample
20q13.2	3.2	Finnish extended families

Abbreviations: DZ, dizygotic; MLOD, multipoint logarithm of odds score.

Table 5 Studies that show linkage in chromosome 3q29 and 7q36 regions

Chromosome	Study group	Number of families	Number of individuals	Trait	Population	LOD
3q29	Francke <i>et al.</i> ²⁷	99	535	Categorized coronary heart disease and myocardial infarction	North Indian origin	2.1
3q29	Kissebah <i>et al.</i> ²⁸	507	2209	Metabolic syndrome component traits	Caucasian	2.4–3.5
3q29	Luke <i>et al.</i> ²⁹	329	1163	BMI	African American	4.3
3q29	Vionnet <i>et al.</i> ³⁰	143	637	Early onset type 2 diabetes	French	4.6 ^a
3q29	Walder <i>et al.</i> ³¹	239	770	BMI	Pima Indians	1.4
3q29	Wu <i>et al.</i> ³²	4021	8042	BMI	Mixed European American, African American and Mexican American	3.4
7q36	Sammalisto <i>et al.</i> ³³	3032	5788	BMI	Mixed European American and African American	2.9 ^b
7q36	Feitosa <i>et al.</i> ³⁴	536	3407	BMI	European American	4.9
7q36	Hsueh <i>et al.</i> ³⁵	28	672	BMI adjusted leptin levels	Amish	1.8
7q36	Pérusse <i>et al.</i> ³⁶	156	521	Abdominal subcutaneous fat	Caucasian	2

Abbreviation: BMI, body mass index. ^aMaximum binomial likelihood, ^bFemale-specific signal.

been shown to regulate cholesterol concentrations in cells and would be an excellent candidate gene for further studies along with *APOD*. Individual cohorts provided evidence of linkage in regions, which have also been previously linked to obesity-related traits: the 2p24^{37–40} area (MLOD = 3.4) in the Dutch cohort and the 20q13^{41–44} area (MLOD = 3.2) in the Finnish cohort. In addition, the Dutch cohort showed significant linkage to the chromosome 16q23 area (MLOD = 3.7), which has been linked to resting energy expenditure.⁴⁵ The linkage studies from individual cohorts with partially overlapping samples have been published before. The linkage study in UK twins did not reveal any significant loci on its own.⁴⁶ The previously published studies in both Dutch twins⁴⁷ and Finnish twins⁴⁸ were considerably smaller than the sample used in this study, and they did not provide significant evidence of linkage. The previously published linkage study in Australian twin families⁴⁹ was slightly smaller (933 families) than the sample used in this study (1153 families), and the results between our Australian sample and previously published data were similar. The linkage studies in Danish and Swedish twins have not been published before. More detailed research on these regions should be conducted within the populations in which they were identified. They might contain some rare alleles that are enriched within these populations but are very rare in other parts of Europe. The strongest evidence for linkage in the combined study came from the DZ twin analysis without the additional family members corroborating that our sampling strategy was successful. Although the possibility of sampling error cannot be excluded, the reason for stronger signal in DZ twin sample was probably the reduction in environmental variance allowing us to show linkage in previously well-replicated regions exemplified by Table 3. Although these regions show evidence of linkage, GWA scans have not yet been able to show evidence of association in all of these regions; GWASs identify only common variants, and current chips do not cover all the existing variations. Therefore, we still need to find ways to

combine the linkage and GWASs to finally find the variants responsible for the linkage signals, which may be caused by genetic markers other than common single-nucleotide polymorphisms.

The contribution of the *FTO* gene to obesity was initially discovered through type 2 diabetes and not by BMI as such. Later studies such as the study by Kring *et al.*⁵⁰ have shown that genetic variants in the *FTO* gene do contribute to fat mass, but the mechanism still remains unclear. Interestingly, this region shows no evidence of linkage thus suggesting that the region does not harbor variants detectable for linkage. It must be realized that the linkage analysis approach detects different variants than does association mapping.⁵¹ The *MC4R* gene has been shown to harbor rare variants which are associated with severe non-syndromic obesity and is the largest known source of contribution in monogenic obesity.^{52,53} There is a linkage peak in the DZ twin sample (MLOD = 1.5) which is 20 cM away from the gene. We plotted all of the GWA findings to our linkage data in Figure 1. One of the closest genes reported in the GWAS in the associated region is marking the position of the association. Interestingly, 4 of the 13 reported GWA loci show linkage >1 in the combined sample including *NPC1*, *LGR4*, *ETV5* and *NCR3*. This would suggest that these regions also harbor rare variants with strong effects. The findings of this study provide further evidence in several regions in the genome. There are obesity genes to be identified under established linkage peaks, which might explain more of the still largely unexplained variance in human BMI.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This project is supported by the European Commission under the program 'Quality of Life and Management of the Living Resources' of 5th Framework Programme (no. QL6-CT-2002-01254), Genome-Wide Analyses of European Twin and Population Cohorts (EU/QLRT-2001-01254), NIH/NHLBI: STAMPEED (Genetics of cardiovascular risk factors in large founder population birth cohorts, 1-R01-HL087679-01), by the Finnish Heart Association, the Sigrid Juselius Foundation and the Academy of Finland Centre of Excellence in Complex Disease Genetics. TwinsUK is supported by the Wellcome Trust, the BBSRC and an NHS NIHR Biomedical research centre Grant to KCL and Guys and St Thomas' foundation hospitals trust. The Swedish twin registry was supported by Swedish Research Council (no. M-2005-1112) and the Swedish Foundation for Strategic Research. The Australian Twin Registry was supported in part by Grants

from NIAAA (USA): AA007535, AA013320, AA013326, AA014041, AA07728, AA10249, AA11998, and NHMRC (Australia): 941177, 951023, 950998, 981339, 241916 and 941944. The Netherlands Twin Register was supported by the Netherlands Heart Foundation (86.083, 88.042, 90.313), the Netherlands Organisation for Scientific Research (904-61-090; SPI 56-464-14192; 480-04-004), Center Medical Systems Biology (NWO Genomics), Genome Scan for Neuroticism (National Institute of Mental Health Grant no. R01 MH059160). Samples were genotyped by the Mammalian Genotyping Service in Marshfield and the Molecular Epidemiology Section, Leiden University Medical Centre. The Danish Twin Registry has been supported by the Danish Medical Research Council, The Danish Diabetes Foundation, the Danish Heart Foundation and the Novo Nordisk Foundation.

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