

The *SLC6A14* gene shows evidence of association with obesity

Elina Suviolahti, ... , Leena Peltonen, Päivi Pajukanta

J Clin Invest. 2003;112(11):1762-1772. <https://doi.org/10.1172/JCI17491>.

Article Aging

In our previous genome-wide scan of Finnish nuclear families, obesity was linked to chromosome Xq24. Here we analyzed this 15-Mb region by genotyping 9 microsatellite markers and 36 single nucleotide polymorphisms (SNPs) for 11 positional and functional candidate genes in an extended sample of 218 obese Finnish sibling pairs (sibpairs) (BMI > 30 kg/m²). Evidence of linkage emerged mainly from the obese male sibpairs, suggesting a gender-specific effect for the underlying gene. By constructing haplotypes among the obese male sibpairs, we restricted the region from 15 Mb to 4 Mb, between markers DXS8088 and DXS8067. Regional functional candidate genes were tested for association in an initial sample of 117 cases and 182 controls. Significant evidence was observed for association for an SNP in the 3'-untranslated region of the solute carrier family 6 member 14 (*SLC6A14*) gene ($P = 0.0002$) and for SNP haplotypes of the *SLC6A14* gene ($P = 0.0007$ – 0.006). Furthermore, an independent replication study sample of 837 cases and 968 controls from Finland and Sweden also showed significant differences in allele frequencies between obese and non-obese individuals ($P = 0.003$). The *SLC6A14* gene is an interesting novel candidate for obesity because it encodes an amino acid transporter, which potentially regulates tryptophan availability for serotonin synthesis and thus possibly affects appetite control.

Find the latest version:

<https://jci.me/17491/pdf>



The *SLC6A14* gene shows evidence of association with obesity

See the related Commentary beginning on page 1633.

Elina Suviolahti,^{1,2} Laura J. Oksanen,^{1,3} Miina Öhman,^{2,4} Rita M. Cantor,^{1,5} Martin Ridderstråle,⁶ Tiinamajja Tuomi,^{3,7} Jaakko Kaprio,^{8,9} Aila Rissanen,¹⁰ Pertti Mustajoki,¹¹ Pekka Jousilahti,¹² Erkki Vartiainen,¹² Kaisa Silander,² Riika Kilpikari,² Veikko Salomaa,¹² Leif Groop,⁶ Kimmo Kontula,³ Leena Peltonen,^{1,2,13} and Päivi Pajukanta¹

¹Department of Human Genetics, University of California, Los Angeles, Los Angeles, California, USA

²Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland

³Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland

⁴Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA

⁵Department of Pediatrics, University of California, Los Angeles, Los Angeles, California, USA

⁶Department of Endocrinology, Wallenberg Laboratory, Lund University, Malmö, Sweden

⁷Folkhälsan Research Institute, Helsinki, Finland

⁸Finnish Twin Cohort Study, Department of Public Health, University of Helsinki, Helsinki, Finland

⁹Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland

¹⁰Obesity Research Unit, Helsinki University Central Hospital, Helsinki, Finland

¹¹Department of Medicine, Peijas Hospital, Vantaa, Finland

¹²Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland

¹³Department of Medical Genetics, University of Helsinki, Helsinki, Finland

In our previous genome-wide scan of Finnish nuclear families, obesity was linked to chromosome Xq24. Here we analyzed this 15-Mb region by genotyping 9 microsatellite markers and 36 single nucleotide polymorphisms (SNPs) for 11 positional and functional candidate genes in an extended sample of 218 obese Finnish sibling pairs (sibpairs) (BMI > 30 kg/m²). Evidence of linkage emerged mainly from the obese male sibpairs, suggesting a gender-specific effect for the underlying gene. By constructing haplotypes among the obese male sibpairs, we restricted the region from 15 Mb to 4 Mb, between markers DXS8088 and DXS8067. Regional functional candidate genes were tested for association in an initial sample of 117 cases and 182 controls. Significant evidence was observed for association for an SNP in the 3'-untranslated region of the solute carrier family 6 member 14 (*SLC6A14*) gene ($P = 0.0002$) and for SNP haplotypes of the *SLC6A14* gene ($P = 0.0007-0.006$). Furthermore, an independent replication study sample of 837 cases and 968 controls from Finland and Sweden also showed significant differences in allele frequencies between obese and non-obese individuals ($P = 0.003$). The *SLC6A14* gene is an interesting novel candidate for obesity because it encodes an amino acid transporter, which potentially regulates tryptophan availability for serotonin synthesis and thus possibly affects appetite control.

J. Clin. Invest. 112:1762-1772 (2003). doi:10.1172/JCI200317491.

Introduction

Obesity poses a serious health problem in the Western societies due to the higher risk for related disorders

Received for publication November 27, 2002, and accepted in revised form October 14, 2003.

Address correspondence to: Päivi Pajukanta, Department of Human Genetics, Gonda (Goldschmied) Neuroscience and Genetics Research Center, 695 Charles E. Young Drive South, David Geffen School of Medicine at UCLA, Los Angeles, California 90095-7088, USA. Phone: (310) 794-9802; Fax: (310) 794-5446; E-mail: ppajukanta@mednet.ucla.edu.

Conflict of interest: The authors have declared that no conflict of interest exists.

Nonstandard abbreviations used: coronary heart disease (CHD); maximum likelihood score (MLS); dizygotic (DZ); single nucleotide polymorphism (SNP); solute carrier family 6 member 14 (*SLC6A14*); sibling pair (sibpair); familial combined hyperlipidemia (FCHL); linkage disequilibrium (LD); National Center for Biotechnology Information (NCBI); identity-by-descent (IBD); 3'-untranslated region (3'-UTR); large neutral amino acid (LNAA).

such as type 2 diabetes, coronary heart disease (CHD), hypertension, osteoarthritis, and certain cancers (1). A significant genetic component has been documented in the etiology of obesity (refs. 2, 3, 4; reviewed in ref. 5). Cases of obesity with a monogenic origin are rare (reviewed in ref. 6), whereas common forms of obesity are most likely caused by multiple genetic and environmental factors, and their interactions.

In Finland, as well as in other Western societies, obesity has become more prevalent during the last three decades (7, 8). Currently, more than 20% of the Finnish population has a BMI greater than 30 kg/m² and can thus be considered obese (9). The Finnish population is a suitable study sample for detecting candidate genes for obesity due to the high prevalence of this disorder and the relative genetic and environmental homogeneity of the population (10).

A locus on chromosome Xq24 was linked to obesity with a maximum likelihood score (MLS) of 3.1 in our

previous genome-wide scan of Finnish obese nuclear families (11). This X-chromosome locus may be of particular interest when searching for genes predisposing to obesity, because obesity and related phenotypes, such as fat distribution, fat percentage, and leptin levels, appear to be influenced by gender (12, 13). Furthermore, quantitative genetic modeling of twin data from monozygotic, same-sex dizygotic (DZ) and opposite-sex DZ pairs indicates that sex-specific genetic influences on BMI are seen in adolescent and young adult population-based twin samples, in addition to genetic factors common to both sexes (14, 15).

None of the other reported genome scans of obesity have revealed a significant linkage to Xq24. However, two separate studies of obesity detected suggestive linkage in this region with lod scores of 1.4 (16) and 2.0 (17). It is worth noting that most genome scans related to obesity have used quantitative traits and did not include the sex chromosomes in their analysis, with the exception of two studies (18, 19). The reason for excluding X-chromosome scan data may be the lack of computer programs for such analyses (20). Interestingly, however, Monaghan et al. reported a woman with Prader-Willi-like syndrome, which features obesity as one of its major symptoms, carrying an Xq23–25 duplication (21).

In this study, we investigated the Xq22–24 region by first haplotyping microsatellite markers and single nucleotide polymorphisms (SNPs) in candidate genes in obese Finnish siblings. After identifying initial evidence for shared haplotypes in a 4-Mb region, we tested SNPs and microsatellites in the region in an initial case-control sample. We found significant evidence of association between obesity and the solute carrier family 6 member 14 (*SLC6A14*) gene ($P = 0.0002$). Importantly, an independent replication sample of 837 cases and 968 controls from Sweden and Finland also showed differences in allele frequencies between obese and non-obese individuals ($P = 0.003$). This is the first report to our knowledge implicating *SLC6A14* in obesity.

Methods

Study samples. The study sample consisted of 218 obese sibling pairs (sibpairs) ($\text{BMI} \geq 30 \text{ kg/m}^2$) from 184 Finnish nuclear families. Of these, 193 sibpairs from 166 families participated in our previous genome scan for obesity (11). In addition, to increase the power to identify an association, we included 25 additional Finnish obese sibpairs ($\text{BMI} \geq 30 \text{ kg/m}^2$) from families ascertained for familial combined hyperlipidemia (FCHL) or low serum HDL levels in the analysis of the stage 2 markers (for definition of stages 1 and 2, see Results). The origin of and the diagnostic criteria for these families have been described earlier (22, 23). These Finnish FCHL and HDL families showed suggestive evidence of linkage with this same X-chromosomal region, with a lod score of 1.5 (23).

For the initial association study, one obese male from each of the 184 original nuclear families, where available, was selected as a case ($n = 117$). A group of unrelated normal-weight Finnish men ($n = 182$) were selected as controls based on their BMI of 27 kg/m^2 or less and age of 40 years or more (Table 1).

An independent replication sample of 837 cases (416 females and 421 males) and 968 controls (381 females and 587 males) was collected from Finland and Sweden to test the associated *SLC6A14* variants for association (Table 1). In detail, 568 obese Finnish cases were recruited from obesity clinics in Helsinki ($n = 207$), from the National FINRISK97 cohort in Finland ($n = 200$) (24), and from the Botnia study ($n = 161$) (25, 26). In addition, a total of 269 Swedish obese subjects were recruited from the obesity clinic in Malmö, Sweden. All the obese subjects from the obesity clinics in Finland and Sweden had a BMI values of 30 kg/m^2 or more and had undergone bariatric surgery or participated in a weight reduction program to treat their obesity. These patients had contacted the obesity clinic on their own initiative and when first contacting the clinic, the patients had BMI values of 40 kg/m^2 or more. The obese cases selected from the National FINRISK97 cohort had BMI values of 35 kg/m^2 or more.

Table 1

Mean age and BMI of subjects in the initial linkage and association study and the analysis of the additional *SLC6A14* SNPs, and in the replication sample

	Affected individuals in the linkage study		Association study		Additional analyzed SNPs	
	Cases	Controls	Controls	Controls (subset)	Cases	Controls
Number (M/F)	398 (157/241)	117 M	182 M	67 M	180 M	180 M
Age (years)	50.7 ± 8.4	50.3 ± 8.1	56.8 ± 8.3	51.3 ± 11.0	51.5 ± 9.3	50.4 ± 9.4
BMI (kg/m ²)	35.8 ± 5.9	36.2 ± 6.1	24.1 ± 2.2	24.1 ± 2.1	38.0 ± 6.6	23.4 ± 1.1
	All		Replication sample		Swedish	
	Cases	Controls	Cases	Controls	Cases	Controls
Number (M/F)	837 (421/416)	968 (587/381)	568 (349/219)	709 (514/195)	269 (72/197)	259 (73/186)
Age (years)	48.5 ± 12.3	49.1 ± 11.3	51.5 ± 10.7	52.1 ± 9.6	41.9 ± 12.2	44.8 ± 14.0
BMI (kg/m ²)	39.8 ± 6.6	23.0 ± 1.4	38.7 ± 6.4	23.4 ± 1.5	41.1 ± 6.9	22.3 ± 1.7

Age and BMI values are given as mean ± standard deviation. M, male; F, female.

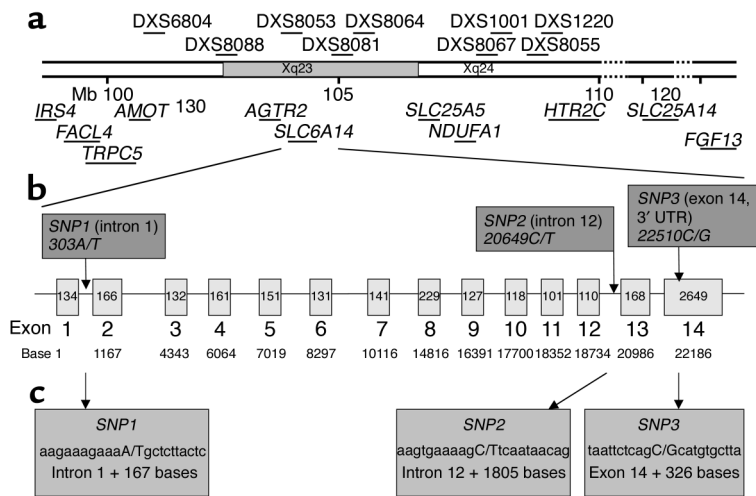


Figure 1
 (a) The genes and microsatellite markers on chromosome Xq22–26 selected for this study. Distances and order are based on the public NCBI and commercial Celera Genomics databases. (b) Structure of the *SLC6A14* gene and locations of the genotyped SNPs. (c) SNPs, their locations in the *SLC6A14* gene, and the surrounding sequence (SNP nucleotides are capitalized).

The obese male cases selected from the Botnia study had BMI values of 30 kg/m² or more and each had at least one additional sibling with a BMI of 30 kg/m² or more. Altogether, 968 lean control subject with BMI values of 25 or less were recruited from the same geographical regions as the obese cases (Table 1).

Of the 49 new *SLC6A14* variants identified in the sequencing analysis, 5 were tested for linkage disequilibrium (LD) and association in 113 of the original male cases of the initial association study and in 67 additional extremely obese males from the obesity clinics in Finland (total *n* = 180). For these analyses, 180 male controls (BMI ≤ 25 kg/m²) were selected from the National FINRISK97 cohort (24). These 180 controls were part of the Finnish replication study sample (see above).

The study protocols were approved by the ethics committees of the participating centers, and each study subject gave a written informed consent.

SNP and marker selection. Microsatellite markers and SNPs were selected in a region spanning 15 Mb on chromosome X (Figure 1a) that has previously been linked to obesity in this same study sample (11). Public (National Center for Biotechnology Information [NCBI]; <http://www.ncbi.nlm.nih.gov>) and commercial (Celera Genomics; <http://www.celera.com>) databases were used to find polymorphisms in the following genes: insulin receptor substrate 4 (*IRS4*), fatty-acid-CoA ligase, long-chain 4 (*FACL4*), transient receptor potential cation channel, subfamily C, member 5 (*TRPC5*), angiotensin receptor 2 (*AGTR2*), solute carrier family 6 member 14 (*SLC6A14*), solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5 (*SLC25A5*), NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1, 7.5kDa (*NDUFA1*), 5-hydroxytryptamine (serotonin) receptor 2C (*HTR2C*),

solute carrier family 25 (mitochondrial carrier, brain), member 14 (*SLC25A14*) and fibroblast growth factor 13 (*FGF13*). These genes were selected based on their potential functional significance or physical position. The physical order of the markers and genes was determined according to the NCBI and Celera Genomics databases.

The SNPs (two to four per gene) were chosen primarily in coding regions and in genomic regions conserved between human and mouse. Comparisons of the genomic sequences (gene ± 10 kb) between these two species were performed using the Pipmaker program (27). We also aimed to cover the genes with evenly spaced SNPs, avoiding gaps larger than 20 kb within a gene. First, samples from eight control individuals of Finnish origin were sequenced to confirm the selected SNPs. Thus, in cases where no heterozygotes were detected among these eight individuals, the particular SNP was discarded. In addition, when adjacent

SNPs were in complete LD in the eight individuals, only one was selected for further studies. The complete list of markers and SNPs genotyped in this study is presented on our website (<http://www.ktl.fi/mols/obe>), and the novel SNPs characterized in this study will be submitted to public databases (NCBI).

Genotyping. Microsatellite markers were genotyped using the ABI Prism 3700 DNA Analyzer and were analyzed with the Genotyper 3.7 software (Applied Biosystems Inc., Foster City, California, USA) as described earlier (11). A pyrosequencing technique was applied for the SNP genotyping using the PSQ96 instrument and the SNP Reagent kit (Pyrosequencing AB, Uppsala, Sweden) (28) in stages 1 and 2 (for definition of these stages, see Results), as well as in the initial association study. The SNPs of the large replication sample as well as the five additional SNPs were genotyped using homogenous Mass Extension reaction on the Mass Array System (Sequenom, San Diego, California, USA), as specified by manufacturer's instructions.

Sequencing of the *SLC6A14* gene. We sequenced the entire *SLC6A14* gene, 800 bp of its putative promoter, and 2 kb downstream from the 3' end of *SLC6A14* (including an adjacent predicted gene, *LOC203413*) in 20 obese male individuals (for selection criteria of the obese individuals, see Results). Sequencing was performed according to the BigDye Terminator Cycle Sequencing protocol (Applied Biosystems Inc.), with minor modifications, and the samples were separated with the automated DNA sequencer ABI 377XL (Applied Biosystems Inc.). Sequence contigs were assembled through use of Sequencher software (Gene Codes Corp., Ann Arbor, Michigan, USA).

Statistical analysis. To test the microsatellite markers and SNPs for linkage, we used a nonparametric affect-

ed sibpair approach using the MAPMAKER/SIBS 2.0 program (29). This method of analysis estimates the proportion of allele sharing on the X chromosome in three kinds of sibpairs separately: male-male, male-female and female-female. For X-chromosome markers, the MLS has only one degree of freedom, because one can only evaluate identity-by-descent (IBD) of chromosomes inherited from the mother's side. Thus, an affected sibpair can share either 0 or 1 allele IBD. The option "all pairs weighted" was chosen to obtain information also from families having more than two obese siblings. Marker allele frequencies were estimated from all individuals using the DOWNFREQ program (30). Haplotypes were constructed using the GENEHUNTER program, version 1.3 (31), for the male sibpairs and for male case and control subjects. Haplotyping of X-chromosome markers in males is unambiguous, since males are hemizygous for the X chromosome.

LD between the marker genotypes was tested using the Genepop v3.1b program, option 2 (<http://wbio-med.curtin.edu.au/genepop>). In this program, one test of association is performed for genotypic LD, and the null hypothesis is that genotypes at one locus are independent from genotypes at the other locus. Then the program creates contingency tables for all pairs of loci and conducts Fisher's exact test for each two-by-two table. In men, the phase information is complete on the X chromosome, and the haploid data could be used reliably to test for LD using this Genepop program.

The DISLAMB program version 2.1 was used to test for association; that is, differences in the distributions of allele frequencies of SNPs and SNP haplotypes between cases and controls (32). A χ^2 test was applied for the diallelic markers, and a likelihood ratio test was used for multiallelic markers.

In the replication study sample, one overall analysis of males and females of Swedish and Finnish origin was performed to test for association with obesity with the multilocus genotypes of the two X-linked SNPs (*SNP2* and *SNP3*), using the option 12 of the Mendel program (33).

In this analysis, empiric *P* values were calculated by permuting the case and control labels within the categories of Swedish males, Swedish females, Finnish males, and Finnish females simultaneously, and combining the data for each permutation to establish a distribution of possible genotypes for cases and controls.

Results

Genotyping the Xq24 markers. We had earlier linked a 15-Mb region on chromosome Xq24 to obesity in a genome-wide scan of Finnish obese nuclear families (11). Here we investigated the region by genotyping additional microsatellite markers and SNPs for regional candidate genes to test them for linkage and association. Our aim was not to cover all the genes located in the 15-Mb region, but to target relevant functional candidate genes for obesity. In addition to the regional markers, we tested two compelling candidate genes, *SLC25A14* and *FGF13*, located 9.1 and 17 Mb, respectively, from the linked region.

In stage 1, we genotyped 36 SNPs and nine microsatellite markers in 100 obese sibpairs and available parents in a 15-Mb region between the *IRS4* and *HTR2C* genes, and in the *SLC25A14* and *FGF13* genes. The complete linkage data are given at our website (www.ktl.fi/mols/obe). We observed two interesting findings in these analyses. First, most of the allele sharing among obese siblings occurred over an 8-Mb region between marker DXS6804 and the *HTR2C* gene. Second, we found that the obese male sibpairs contributed most to the linkage signal in this region (Table 2).

In stage 2, the genotyping was further extended to 118 additional sibpairs for the nine multiallelic markers and 17 SNPs in the *AGTR2*, *SLC6A14*, *SLC25A5*, *NDUFA1*, and *HTR2C* genes located in the 8-Mb region where most of the allele sharing occurred in stage 1. Results from the combined analysis of the stage 1 and stage 2 study samples provided some evidence for linkage to this region, but did not narrow down the region (Table 2).

Haplotyping. Because most of the linkage evidence for the SNPs and markers in the Xq22–24 region emerged from obese male sibpairs, we analyzed haplotypes of all male sibpairs in the study to investigate whether any were shared by the obese male sibpairs. We detected three putative shared haplotypes in the *SLC6A14* gene. A total of 16 families out of 26 shared the same 303A-20649C-22510G haplotype of the *SLC6A14* SNPs, three families shared the 303A-20649T-22510C haplotype and two families shared the 303T-20649C-22510G haplotype (Figure 2). These shared haplotypes of the *SLC6A14* gene partially extended to the neighboring markers (DXS8053 and DXS8081) and genes (*AGTR2*, *SLC25A5* and *NDUFA1*). Interestingly, two families shared a 3-Mb haplotype from marker DXS8053 to the *NDUFA1* gene, and three families shared a 1-Mb haplotype from the *AGTR2* gene to marker DXS8081 (Figure 2). There was also a shared haplotype (-2328C-(-)935C-2028C-3169A) within the *SLC25A5* gene, located 1.1 Mb from the *SLC6A14* gene (the minus sign before the nucleotide indicates that the SNP is located 5' from the transcription starting site). This haplotype was detected in 14 families, but it was only partially shared among the *SLC6A14* 303A-20649C-22510G haplotype carriers (Figure 2). In summary, the haplotype data suggested sharing within the *SLC6A14* gene, although it was not specific to this gene. Subsequently, we selected 11 informative SNPs for the *SLC6A14* gene and for its two neighboring genes (*AGTR2* and *SLC25A5*) and also genotyped three microsatellite markers (DXS8081, DXS8053 and DXS8064) located in this region for an association analysis.

Initial association study. The obese nuclear families of this study are not optimal for a family-based association analysis, since parental genotype information is often missing. To search for association with a more powerful approach, we used a case-control sample in the region showing initial evidence of shared haplotypes. Three SNPs of the *SLC6A14* gene were genotyped

Table 2

Single-point MLS of microsatellite markers and SNPs genotyped in stages 1 and 2 of the linkage and haplotype analysis for all study subjects and for male pairs

Marker		MLS for all study subjects (males only) stage 1	MLS for all study subjects (males only) stage 2	Heterozygosity	Intermarker distance (Mb)
DXS6804		1.15 (1.15)	0.14 (0.14)	0.68	
DXS8088		0.07 (0.07)	0.0	0.67	+ 1.2
AGTR2	SNP1	0.60 (0.49)	0.19 (0.10)	0.50	+ 0.8
	SNP2	0.52 (0.32)	0.24 (0.04)	0.50	
	SNP3	1.43 (0.43)	1.18 (0.32)	0.45	
	SNP4	2.30 (1.30)	1.50 (0.64)	0.49	
DXS8053		1.46 (0.70)	1.03 (0.44)	0.49	+ 0.3
SLC6A14	SNP1	1.27 (1.08)	1.15 (0.91)	0.35	+ 0.03
	SNP2	0.11 (0.0)	0.22 (0.21)	0.40	
	SNP3	0.47 (0.0)	0.62 (0.31)	0.47	
DXS8081		0.75 (0.75)	0.29 (0.21)	0.63	+ 0.5
DXS8064		0.86 (0.86)	0.57 (0.57)	0.46	+ 0.8
SLC25A5	SNP1	0.19 (0.07)	0.43 (0.43)	0.48	+ 1.3
	SNP2	0.53 (0.43)	0.19 (0.19)	0.36	
	SNP3	0.80 (0.42)	0.29 (0.19)	0.35	
	SNP4	0.67 (0.29)	0.28 (0.19)	0.35	
NDUFA1	SNP1	0.12 (0.08)	0.71 (0.65)	0.50	+ 0.4
	SNP2	0.25 (0.05)	0.17 (0.16)	0.07	
DXS8067		0.68 (0.68)	1.44 (1.44)	0.63	+ 0.3
DXS1001		0.96 (0.68)	1.10 (0.91)	0.81	+ 0.5
DXS8055		0.02 (0.02)	0.11 (0.11)	0.57	+ 0.6
DXS1220		1.93 (1.93)	0.21 (0.21)	0.55	+ 0.05
HTR2C	SNP1	0.55 (0.48)	0.11 (0.10)	0.31	+ 0.5
	SNP2	0.68 (0.48)	0.10 (0.10)	0.41	
	SNP3	0.81 (0.06)	0.40 (0.03)	0.17	
	SNP4	0.87 (0.0)	1.16 (0.05)	0.17	

MLS scores greater than 1.0 are indicated in bold.

in an initial case-control study sample of 182 independent controls and 117 obese males (the first obese male genotyped from each of the families). We only included males in this initial case-control study, as the initial linkage evidence was mostly derived from male-male pairs and the evidence for haplotypes was detected in males. In these analyses, the observed allele frequencies differed significantly between cases and controls, producing a P value of 0.0002 for the *SNP3* located in the 3'-untranslated region (3'-UTR) (22510C/G) of the *SLC6A14* gene (allele frequencies of 0.70 versus 0.46 for cases versus controls for *SNP3* G allele; Table 3). Suggestive evidence for association ($P = 0.07$) was also detected for the *SNP2* in intron 12 (20649C/T) of the *SLC6A14* gene (0.72 versus 0.61 for cases versus controls for *SNP2* C allele; Table 3). Interestingly, the more frequent allele of both SNPs was associated with obesity. When these two intragenic SNPs were combined into haplotypes, the difference in allele frequencies remained significant ($P = 0.0007$), as it did when all three SNPs of the *SLC6A14* gene were combined ($P = 0.006$; Table 3). The obesity-associated haplotype alleles (20649C-22510G and 303A-20649C-22510G) were also the major alleles in the study sample (frequencies of 0.70 and 0.52 for cases and 0.46 and 0.33 for controls, respectively).

Because haplotype sharing was not specific to the *SLC6A14* gene (Figure 2), we also genotyped eight SNPs in the neighboring genes, *AGTR2* and *SLC25A5*, as well as three microsatellite markers, DXS8088, DXS8081, and DXS8064. The allele frequencies for the SNPs in the *AGTR2* and *SLC25A5* genes did not differ between male cases ($n = 117$) and male controls ($n = 67$) (Table 4). No additional controls were genotyped. The allele frequencies of marker DXS8081, located in the vicinity of the *SLC6A14* gene, showed a small difference between cases and controls ($P = 0.05$) (Table 4), supporting the evidence obtained regarding *SLC6A14* in the association analysis.

Replication study. The obesity-associated *SNP2* and *SNP3* were genotyped in an independent replication sample of 837 cases (416 females and 421 males) and 968 controls (381 females and 587 males) from Finland and Sweden. Again, significant differences in allele frequencies were detected for *SNP3* between the two groups ($P = 0.003$), with the C allele being more common among obese subjects (allele frequencies of 0.49 in cases and 0.43 in controls; Table 5). For *SNP2*, the T allele was more frequent in cases than in controls, but the difference was not statistically significant (0.38 versus 0.35 for cases versus controls for T allele; $P = 0.08$). Furthermore, in one overall analysis of multilocus

Family	Individual	DXS8088	AGTR SNP4	AGTR SNP3	AGTR SNP2	AGTR SNP1	DXS8053	SLC6A14 SNP1	SLC6A14 SNP2	SLC6A14 SNP3	DXS8081	DXS8064	SLC25A5 SNP1	SLC25A5 SNP2	SLC25A5 SNP3	SLC25A5 SNP4	NDUFA1 SNP1	NDUFA1 SNP2	DXS8067	DXS1001	
1	1	8	T	G	G	A	2	A	C	G	4	7	C	C	C	A	G	T	6	1	Allele sharing within family
1	2	8	T	G	G	A	2	A	C	G	4	7	T	T	T	G	C	T	6	0	
2	1	8	T	G	T	A	6	A	C	G	5	9	T	C	C	A	G	T	6	1	Allele sharing between families
2	2	9	T	G	G	A	4	A	C	G	6	6	T	T	T	G	G	T	7	1	
3	1	7	T	G	T	A	6	A	C	G	4	7	T	T	T	G	G	T	6	1	Allele sharing between families
3	2	7	T	G	T	A	6	A	C	G	4	7	T	T	T	G	G	T	6	0	
4	1	6	T	G	T	A	6	A	C	G	5	6	C	C	C	A	G	T	8	2	Allele sharing between families
4	2	6	T	G	T	A	6	A	C	G	5	6	C	C	C	A	G	T	8	2	
5	1	8	C	A	G	G	0	A	C	G	5	5	C	C	C	A	G	T	8	4	
5	2	8	C	A	G	G	6	A	C	G	5	5	C	C	C	A	G	T	8	4	
6	1	7	C	A	G	G	6	A	C	G	5	7	T	C	C	A	G	T	7	1	
6	2	7	C	A	G	G	6	A	C	G	5	7	T	C	C	A	G	T	7	1	
7	1	8	C	A	G	G	6	A	C	G	6	7	C	C	C	A	C	0	4	0	
7	2	8	C	A	G	G	6	A	C	G	6	7	C	C	C	A	C	0	4	1	
7	3	8	C	A	G	G	6	A	C	G	6	7	C	C	C	A	C	0	4	0	
8	1	6	C	A	G	G	4	A	C	0	6	7	T	T	T	G	C	T	7	5	
8	2	6	C	A	G	G	4	A	C	G	6	7	T	T	T	G	C	T	7	5	
8	3	6	C	A	G	G	4	A	C	G	6	7	T	T	T	G	C	T	7	5	
9	1	9	T	G	T	A	4	A	C	G	6	7	T	T	T	G	C	T	6	4	
9	2	9	T	G	T	A	4	A	C	G	6	0	T	T	T	G	C	T	6	4	
10	1	8	T	G	T	A	4	A	C	G	6	9	C	C	C	A	G	T	6	3	
10	2	8	T	G	T	A	4	A	C	G	6	9	C	C	C	A	G	T	6	3	
11	1	7	T	G	T	A	4	A	C	G	6	6	T	C	C	A	G	T	9	2	
11	2	7	T	G	T	A	4	A	C	G	6	6	T	C	C	A	G	T	9	2	
12	1	7	0	0	0	0	4	A	C	G	6	7	0	0	0	0	G	0	6	1	
12	2	7	0	0	0	0	4	A	C	G	6	7	0	0	0	0	G	0	6	1	
13	1	8	C	A	G	G	4	A	C	G	5	8	T	T	T	G	G	T	6	5	
13	2	8	C	A	G	G	4	A	C	G	5	8	T	T	T	G	G	T	6	5	
14	1	8	T	G	G	A	0	A	C	G	5	7	C	C	C	A	C	T	6	0	
14	2	8	T	G	G	A	4	A	C	G	5	7	C	C	C	A	C	T	6	4	
15	1	8	C	A	G	G	4	A	C	G	4	7	C	C	C	A	C	T	6	6	
15	2	6	T	G	T	A	4	A	C	G	4	7	T	T	T	G	G	T	6	4	
16	1	9	T	G	G	0	4	A	C	G	4	7	C	C	C	A	G	T	6	6	
16	2	7	C	A	G	G	4	A	C	G	5	8	C	C	C	A	G	T	6	6	
17	1	6	C	A	G	G	4	A	T	C	4	7	T	C	C	A	G	T	6	0	
17	2	9	C	A	G	G	4	A	T	C	4	7	T	C	C	A	G	T	6	0	
18	1	8	C	A	G	G	4	A	T	C	5	7	C	C	C	A	C	T	6	3	
18	2	8	C	A	G	G	4	A	T	C	5	7	C	C	C	A	C	T	6	3	
19	1	6	C	A	G	G	4	A	T	C	6	7	C	C	C	A	C	T	6	3	
19	2	6	C	A	G	G	4	A	T	C	6	7	C	C	C	A	C	T	6	3	
20	1	9	T	G	G	A	4	T	C	G	6	7	C	C	C	A	G	T	6	5	
20	2	9	T	G	G	A	4	T	C	G	6	7	C	C	C	A	G	T	6	5	
20	3	9	T	G	G	A	4	T	C	G	6	7	C	C	C	A	G	T	6	5	
21	1	Z	T	G	G	A	4	T	C	G	4	6	C	C	C	A	C	T	5	4	
21	2	Z	T	G	G	A	4	T	C	G	4	6	C	C	C	A	C	T	5	4	
21	3	9	T	G	T	G	4	T	C	G	8	8	0	C	C	A	C	T	5	6	
22	1	6	T	G	G	A	5	T	T	C	6	7	C	C	C	A	G	T	6	1	
22	2	6	T	G	G	A	5	T	T	C	6	7	C	C	C	A	G	T	6	1	
23	1	7	C	A	G	G	4	A	C	G	6	7	C	C	C	A	C	T	6	4	
23	2	8	C	A	G	G	4	A	T	C	6	7	C	C	C	A	G	T	8	1	
24	1	6	T	G	T	A	0	T	C	G	8	7	C	C	C	A	G	T	5	7	
24	2	8	T	G	T	A	6	A	C	G	5	0	C	C	C	A	G	T	5	0	
25	1	8	T	G	T	A	4	A	C	G	5	8	C	C	C	A	C	T	6	4	
25	2	9	T	G	G	A	4	A	T	C	4	7	C	C	C	A	G	T	6	0	
26	1	6	T	G	G	A	4	A	C	G	4	7	T	C	C	A	C	T	7	1	
26	2	7	0	A	G	G	6	A	T	C	6	7	C	C	C	A	C	T	6	1	

Figure 2

Haplotype sharing on Xq23-24 among male sibpairs within families and between families. The major haplotype of the *SLC6A14* gene (303A-20649C-22510G) is shared by 16 families and the shared haplotype partially extends to the neighboring markers.

genotypes of both tested X-linked SNPs (*SNP2* and *SNP3*) in males and females of Swedish and Finnish origin, association with obesity was observed ($P < 0.05$). This analysis was performed using option 12 of the Mendel program (33). These analyses indicate that among all the possible genotype distributions, the probability of observing one more associated than the one seen for these data is 5%. This association may result from the individual contributions of the four

ethnic-gender combinations. Importantly, this overall analysis provides power to detect associations that may not be identified in the smaller groups (Table 1). Thus, combined analysis of both SNPs also provided evidence for association in the replication study sample.

When the SNPs were investigated separately in both sexes, the females were found to contribute most to the observed association ($P = 0.006$) for *SNP3*, with allele frequencies of 0.48 in cases ($n = 416$) and 0.41

Table 3

Allele frequencies of the SNPs and SNP haplotypes of the *SLC6A14* gene in the initial case-control analysis.

Variant in the <i>SLC6A14</i> gene	Allele frequency		P
	Obese (n = 117)	Controls (n = 182)	
303A/T = <i>SNP1</i>	0.76/0.24	0.73/0.27	NS
20649C/T = <i>SNP2</i>	0.72/0.28	0.61/0.39	NS (0.07)
22510C/G = <i>SNP3</i>	0.30/0.70	0.54/0.46	0.0002
Haplotypes of the <i>SNP1</i>, <i>SNP2</i>, and <i>SNP3</i>			0.006
303A-20649C-22510G	0.52	0.33	
303T-20649C-22510G	0.17	0.13	
303A-20649C-22510C	0.04	0.10	
303T-20649C-22510C	0.01	0.02	
303A-20649T-22510C	0.21	0.29	
303T-20649T-22510C	0.05	0.12	
Haplotypes of the <i>SNP2</i> and <i>SNP3</i>			0.0007
20649C-22510G	0.70	0.46	
20649C-22510C	0.05	0.13	
20649T-22510C	0.26	0.41	

SNP1 is located in Intron 1; *SNP2*, in intron 12; and *SNP3*, in the noncoding region of exon 14. Significant P values are indicated in bold.

in controls (n = 381) for the C allele. This difference was present in both Finnish and Swedish females (P = 0.08 and 0.03, respectively). For *SNP2*, the difference in allele frequencies was not significant for females and males separately.

Because of the X-inactivation in females, we analyzed the females homozygous for either of the alleles in *SNP2* and *SNP3* separately. For *SNP3*, CC-genotype carriers were more prevalent in the obese group (P = 0.009), and a marginal difference was detected in the Swedish females alone (P = 0.10 and 0.04 for Finnish and Swedish, respectively). No significant difference was detected for *SNP2*. To see if the haplotype might be acting in a recessive fashion, we tested whether the proportion of females homozygous for the *SNP2* and *SNP3* (i.e., genotype combinations of *SNP2CC-SNP3CC*,

SNP2CC-SNP3GG, *SNP2TT-SNP3GG*, and *SNP2TT-SNP3CC*) was significantly different in the case and control groups. No difference was observed.

SLC6A14 gene variants. The entire *SLC6A14* gene, 800 bp of its putative promoter, and 2 kb downstream from the 3' end of *SLC6A14* (including an adjacent predicted gene, *LOC203413*; GenBank accession number XM_117548) were sequenced in 20 obese male individuals. These obese males were selected from the families sharing the allele with another obese male sibling at the Xq24 locus as follows: 17 of the individuals sequenced for the region shared the obesity-associated haplotype for *SNP2* and *SNP3* in the *SLC6A14* gene, and 3 individuals shared the allele more prevalent among the lean controls. Our sequence analysis of the *SLC6A14* gene and its putative 5' promoter revealed 49 variants in addition to the three SNPs genotyped earlier (Table 6). Two of the variants resided in the putative promoter; two, in the coding region; four, in the 3'-UTR; and 41, in the intronic regions. None of the variants changed an amino acid. In the predicted gene *LOC203413*, we found one SNP in the 3'-UTR of the gene and an insertion of four bases in intron 1 (Table 6). Thus a total of 54 variants were identified for the *SLC6A14* region, including the three SNPs genotyped earlier, and 30 of these variants were novel, not found in the public (NCBI, LocusLink) or commercial (Celera) SNP databases previously (Table 6). A total of 23 of 30 novel variants were seen only in the group of obese males sharing the obesity-associated haplotype for *SNP2* and *SNP3* in the *SLC6A14* gene. Five of the variants (-715C/G and -298A/G in the putative *SLC6A14* promoter, 3401C/T in intron 2, 23041C/T and 24447A/G in exon 14) were tested for association in a subset of obese (n = 180) and lean (n = 180) Finnish males (see Methods). The allele frequencies for SNP 23041C/T in exon 14 differed between cases and controls (0.70 versus 0.80 for the C allele; P = 0.05) (Table 7). This variant is located 530 bp downstream from *SNP3*. No differences were observed for the remaining four SNPs (Table 7).

Table 4

Allele frequencies of the SNPs of the *AGTR2* and *SLC25A5* genes and of microsatellite markers in the initial case-control analysis.

Gene	Variant/allele	Allele frequency		P
		Obese (n = 117)	Initial controls (n = 67)	
<i>AGTR2</i>	190A/G = <i>SNP1</i>	0.48/0.52	0.41/0.59	NS
	2822G/T = <i>SNP2</i>	0.65/0.35	0.63/0.37	NS
	2828A/G = <i>SNP3</i>	0.43/0.57	0.52/0.48	NS
	5747C/T = <i>SNP4</i>	0.44/0.56	0.51/0.49	NS
<i>SLC25A5</i>	-2328C/T = <i>SNP1</i>	0.61/0.39	0.50/0.50	NS
	-935C/T = <i>SNP2</i>	0.78/0.22	0.72/0.28	NS
	2028C/T = <i>SNP3</i>	0.78/0.22	0.73/0.27	NS
	3169A/G = <i>SNP4</i>	0.78/0.22	0.74/0.26	NS
DXS8053	2/3/4/5/6	0.04/0.02/0.66/0.03/0.25	0.07/0/0.61/0.06/0.26	NS
DXS8081	4/5/6/8	0.30/0.22/0.47/0.01	0.42/0.08/0.45/0.05	0.05
DXS8064	5/6/7/8/9	0.01/0.17/0.73/0.07/0.02	0/0.15/0.62/0.16/0.07	NS

Significant P value is indicated in bold.

Table 5

Allele frequencies of the SNP2 and SNP3 of the *SLC6A14* gene in the replication sample.

The variant in the <i>SLC6A14</i> gene	Allele frequency		P
	Obese (n = 837)	Controls (n = 968)	
All individuals			
20649C/T = SNP2	0.62/0.38	0.65/0.35	NS (0.08)
22510C/G = SNP3	0.49/0.51	0.43/0.57	0.003
Females			
20649C/T = SNP2	0.63/0.37	0.67/0.33	NS
22510C/G = SNP3	0.48/0.52	0.41/0.59	0.006
Males			
20649C/T = SNP2	0.60/0.40	0.63/0.37	NS
22510C/G = SNP3	0.50/0.50	0.45/0.55	NS
Haplotypes of the SNP 2 and SNP 3 in males ³			NS
20649C-22510G	0.50	0.55	
20649C-22510C	0.10	0.08	
20649T-22510C	0.40	0.37	

Significant P values are indicated in bold.

LD structure in the *SLC6A14* region. LD across the *SLC6A14* locus was investigated separately in men and women because the haplotypes can be unequivocally identified on male X chromosomes. In men, the two SNPs (*SNP2* and *SNP3*) that showed evidence for association with obesity, as well as the five additional SNPs that were also tested for association (Table 7), were tested for LD in a subset of obese ($n = 180$) and lean ($n = 180$) Finnish males (see Methods). *SNP2* and *SNP3* were in tight but not complete LD ($P < 0.00001$). Both of these SNPs were also in LD with the two SNPs in exon 14 ($P < 0.00001$). All four SNPs are located within a 4-kb region (Table 6). The LD decreases with the three SNPs in the putative promoter and in intron 2 ($P > 0.003$), located 17–21 kb from *SNP2* (Table 6). In addition, microsatellites DXS8081 and DXS8064, which were the closest markers (0.5 Mb and 1.4 Mb) to the SNPs in exon 14 of *SLC6A14*, were tested for LD in the original sample set of 117 male cases and 67 male controls. DXS8081 was found to be in weak LD with *SNP2* and *SNP3* (P values of 0.004–0.01) and also showed some evidence for association ($P = 0.05$). No evidence for LD or association was detected with marker DXS8064. To summarize the results of the LD analyses in men, the strongest LD was found between the SNPs in the 3' end of the *SLC6A14* gene. There was no difference between the male cases and controls in the extent of LD.

In the 416 female cases and 381 female controls of the replication sample, the two SNPs (*SNP2* and *SNP3*) were also found to be in tight but not complete LD ($P < 0.00001$). The genotype analysis also showed that combinations of the *SNP2CT-SNP3GG* (2 females) and *SNP2TT-SNP3GG* (0 females) genotypes

are almost completely missing, suggesting that *SNP2T* does usually not occur on the same chromosomal DNA strand with *SNP3G*.

Discussion

In the present study, we investigated a region on chromosome Xq22–24 that we had previously linked to obesity in Finnish obese nuclear families (11). We have now restricted the linked region from 15 Mb to 4 Mb with linkage and haplotype analyses. Furthermore, we detected significant associations between obesity and SNPs in exon 14 and intron 12 ($P = 0.0002$ and 0.07) and SNP haplotypes ($P = 0.0007$ and 0.006) of the *SLC6A14* gene in an initial case-control sample. Importantly, an independent replication sample of 837 cases and 968 controls from Finland and Sweden also showed differences in allele frequencies for *SNP3* between cases and controls ($P = 0.003$), and furthermore, a combined analysis of *SNP2* and *SNP3* provided evidence for association ($P < 0.05$) in this replication sample.

This is the first report to our knowledge implying that the *SLC6A14* gene (also known as Na- and Cl-dependent neutral and cationic amino acid transporter B⁰⁺) could be associated with obesity and body weight regulation. The *SLC6A14* gene presents a novel candidate gene for obesity based on its location as well as its function. It encodes a Na- and Cl-dependent membrane protein that transports neutral and cationic amino acids across the plasma membrane (34). Certain amino acid derivatives are essential in appetite regulation. For example, serotonin (5-hydroxytryptamine), that is a neurotransmitter increasing satiety, is synthesized from tryptophan. The rate of serotonin synthesis depends on tryptophan availability in relation to the large neutral amino acids (LNAA) (35), because tryptophan competes with other amino acids for uptake across the blood-brain barrier (36). Thus, factors affecting amino acid absorption from the intestinal tract or transportation across the plasma membranes into the target cell could potentially affect the tryptophan availability for serotonin synthesis, and further the appetite control. It has been shown that *SLC6A14* has a high affinity to tryptophan as well as to some other neutral and cationic amino acids (34). Interestingly, Roca et al. reported a lower tryptophan/LNAA ratio in subjects with high BMI, and this difference was more pronounced in men than in women (37). In addition, both tryptophan hydroxylase, which is a rate-limiting enzyme in serotonin synthesis, and *SLC6A14* are expressed in the pituitary gland.

Gender-specific differences in the prevalence of severe obesity (BMI > 35 kg/m²) are well established. Females are more often affected than males in Finland (38). Even more pronounced are the differences between men and women in obesity-related features such as fat percentage and fat distribution, which are influenced by hormonal factors such as sex steroids and leptin, or differences in fatty acid oxidation and metabolism (12, 13). However, the variants of an X-chromosome gene

Table 6The variants identified in the sequencing analysis of the *SLC6A14* gene and the adjacent predicted gene, *LOC203413*.

Variants in the <i>SLC6A14</i> gene	Nucleotide position from the start site of the <i>SLC6A14</i> gene	Variant	NCBI SNP Cluster ID Cluster ID and/or Celera SNP ID
-715	-715	C/G	(novel)
-298	-298	A/G	(novel)
Intron 1 + 167 = SNP1 in Table 3	303	A/T	rs2312054, CV15979351
Exon 2 + 36 (Gly-Gly)	1,204	A/T	(novel)
Intron 2 + 398	1,733	A/G	(novel)
Intron 2 + 456	1,791	C/T	(novel)
Intron 2 + 1,341	2,676	A/G	(novel)
Intron 2 + 2,066	3,401	C/T	rs5905284, CV343447
Intron 2 + 2,271	3,605	A/G	(novel)
Intron 2 + 2,406	3,740	A/G	(novel)
Intron 3 + 165	4,641	C/T	rs5905285, CV2521237
Intron 3 + 890	5,366	A/G	rs5905176, CV2521238
Intron 3 + 1,412	5,888	C/T	rs5905177, CV26290629
Intron 3 + 1,547	6,023	C/T	(novel)
Intron 5 + 998	8,167	C/T	(novel)
Intron 5 + 1041	8,200	A/C	(novel)
Intron 6 + 811	9,243	C/T	rs2312055, CV15979352
Intron 6 + 843	9,273	A/G	(novel)
Intron 6 + 912	9,342	C/G	rs2312056, CV15979362
Intron 6 + 998	9,428	C/T	rs2312057, CV15979363
Intron 6 + 1,023	9,453	C/T	rs4824323
Intron 6 + 1,027	9,457	C/T	rs4824324
Intron 6 + 1,069	9,499	A/G	rs4824358
Intron 6 + 1,074	9,504	A/G	rs4824359
Intron 6 + 1,080-1,081	9,510	AT/TC	(novel)
Intron 6 + 1,085	9,515	A/C	(novel)
Intron 7 + 565	10,825	A/C	(novel)
Intron 7 + 587	10,846	A/G	(novel)
Intron 7 + 1,618	11,877	G/T	(novel)
Intron 7 + 1,810	12,070	C/G	(novel)
Exon 8 + 144 (Ser-Ser)	14,962	C/T	(novel)
Intron 8 + 252	15,299	C/G	(novel)
Intron 8 + 641	15,688	A/T	(novel)
Intron 8 + 1,049	16,096	A/G	(novel)
Intron 9 + 95	16,614	C/G	(novel)
Intron 9 + 713	17,232	G/T	rs4824325
Intron 10 + 69	17,890	C/G	CV2474483
Intron 10 + 174	17,995	C/T	(novel)
Intron 11 + 82	18,537	A/G	rs5905287
Intron 12 + 67	18,912	A/G	(novel)
Intron 12 + 184	19,029	A/G	(novel)
Intron 12 + 224	19,068	A/T	(novel)
Intron 12 + 281	19,125	A/C	rs5905288
Intron 12 + 746	19,590	C/T	rs5905178
Intron 12 + 1805 = SNP2 in Table 3	20,649	C/T	rs2071877, CV2474484
Intron 13 + 170	21,324	C/G	rs2032525, CV2474485
Intron 13 + 837	21,991	A/C	rs2011198, CV8789802
Exon 14 + 325 (3'-UTR) = SNP3 in Table 3	22,510	C/G	rs2011162, CV8789801
Exon 14 + 855 (3'-UTR)	23,040	C/T	rs5905289
Exon 14 + 1,750 (3'-UTR)	23,935	C/T	rs5905179
Exon 14 + 1,996 (3'-UTR)	24,181	A/C	(novel)
Exon 14 + 2,262 (3'-UTR)	24,447	A/G	CV2474486
Variants in the <i>LOC203413</i> (predicted gene)			
Exon 2 + 297 (3'-UTR)	25,089	C/T	(novel)
Intron 1 + 333	25,822	AAAG/-	(novel)

The SNPs showing initial evidence of association (Table 3) are indicated in bold. A minus sign before the nucleotide indicates that the SNP is located 5' from the transcription starting site. ID, identifier.

predisposing to obesity may also be involved in the development of these gender-specific differences, as implied by twin data (14, 15).

In this study, we utilized the strategy of haplotype mapping and identified a candidate region from which we selected a denser set of SNPs that were genotyped to search for association. A similar approach was previously successful in mapping susceptibility loci for com-

plex disorders such as inflammatory bowel disease (39, 40). Previously, we found evidence for linkage between obesity and the region on Xq24 with a single-point MLS of 3.1 with marker DXS6804 (11) located 2.3 Mb from the region showing evidence for association in this study. However, when the study sample was extended and the microsatellite markers were reanalyzed with newer technology using the ABI Prism 3700

capillary instrument instead of the gel-based ABI 377XL automated DNA sequencer used in the previous study, the highest single-point MLS of 2.3 was obtained with the *SNP1* in the *AGTR2* gene located 0.33 Mb from the associated region. The linkage signal on chromosome X emerged largely from the obese male sibpairs (Table 2), which led us to hypothesize that they would provide the best sample for the haplotype analysis. Additionally, since males are hemizygous for X-chromosomes, no bias is introduced to male haplotypes because of missing parental genotype information. Analyses revealed shared haplotypes around the *SLC6A14* gene. We tested three SNPs of the *SLC6A14* gene for association in an initial case-control study sample. These three SNPs are located in intron 1 (303A/T = *SNP1*), in intron 12 (20649C/T = *SNP2*), and in the noncoding region of exon 14 (22510C/G = *SNP3*) (Table 6). The last two SNPs in the 3'-end of the *SLC6A14* gene (*SNP2* and *SNP3*) were associated with obesity, as was the SNP haplotype of this gene (Table 3). The obesity-associated haplotype was also the most frequent haplotype in the study sample (frequencies of 0.52 in cases versus 0.33 in controls, respectively).

Next, the two SNPs of the *SLC6A14* gene showing evidence of association in the initial association analysis were further tested for association in an independent replication sample of 837 cases and 968 controls from Finland and Sweden, including both females and males. Again significant differences in allele frequencies were observed with *SNP3* ($P = 0.003$) (Table 7). We also observed that the obesity-associated allele in the replication study was opposite to the one identified in the initial study (Tables 3 and 5). This may reflect the different haplotype background of the two study populations. It is also possible that the actual causative variant(s) are in LD with the associated variants detected here. Although both Finnish study samples (the original and replication) were collected mostly from the southern part of Finland, the birthplaces of the mothers in the initial study were clustered in the eastern parts of Finland, in the Savo and Karelia regions. The replication sample with its relatively large number of individuals is probably not as regionally biased and represents the general population. Furthermore, the association in the replication study emerged mainly from women. This may reflect two things. On the one hand, the size of the replication study sample should have enabled us to detect differences of this magnitude in allele frequencies (Table 5). On the other hand, our original study sample may not have provided enough power to detect evidence of linkage and shared haplotypes especially in females, because of its small size (184 nuclear families with 80 female sibpairs) and small amount of parental genotype data ($n = 59$) available, whereas in males the haploid X-chromosomal data easily provided this crucial information. It is also possible that some unknown gender-specific factors as well as random X-inactivation affect the results, thus partially explaining these allelic differences.

Based on the current data, we cannot confirm whether the associated variants in the *SLC6A14* gene actually predispose to obesity or if the functional variants are in LD with these and located in some other genes close to the *SLC6A14* gene, although it is unlikely that the LD would reach very far from the variants studied here, especially in the large replication study sample. Our results are promising, as the association was replicated in an independent study sample. We also sequenced the entire *SLC6A14* gene and the adjacent predicted gene, *LOC203413*. Although a total of 54 variants were identified (Table 6), none of the variants has an obvious effect on the protein function (i.e., amino acid exchange or truncated product). Thus, none of the SNPs represents an obvious causative variant such as a nonsense or missense mutation, suggesting that they may instead serve as other regulatory or splicing elements for the *SLC6A14* gene or be in LD with an unidentified coding variant of a gene located nearby. Future studies in other obesity samples, preferably linked to this region, are warranted to address the question of the causative variant(s) and could be undertaken prior to functional studies. Other populations could further help restrict the region due to their possibly different underlying LD structure and also because functional studies of noncoding variants are very challenging and should be restricted to a minimum number of SNPs. Furthermore, it is possible that the predisposing variant in the promoter, untranslated region, or intron has a small but significant effect for example on the transcription rate. The predisposing allele could also represent a combination of different variants. However, these effects are difficult to tackle without thorough association studies in several populations.

Interestingly, a recent genome-wide scan for CHD in Finns revealed a significant evidence for linkage to this same region on Xq23–26, with a two-point lod score of 3.5 (41). Furthermore, the results of that study suggested an initial association with marker DXS8053 ($P = 0.007$), located in the immediate vicinity of the *SLC6A14* gene (30 kb). Since overweight predisposes to CHD, and CHD patients are often obese, it is tempting

Table 7

Results of the association analysis of the five additional *SLC6A14* variants.

The variant in the <i>SLC6A14</i> gene	Allele frequency		<i>P</i>
	Obese ($n = 180$)	Controls ($n = 180$)	
-715C/G	0.72/0.28	0.73/0.27	NS
-298A/G	0.96/0.04	0.97/0.03	NS
3,401C/T (intron 2 + 2,066)	0.26/0.74	0.28/0.72	NS
2,3040C/T (exon 14 + 855)	0.70/0.30	0.80/0.20	0.05
2,4447A/G (exon 14 + 2262)	0.80/0.20	0.81/0.19	NS

Significant *P* value is indicated in bold. A minus sign before the nucleotide indicates that the SNP is located 5' from the transcription starting site.

to hypothesize that allelic variants of the same gene may predispose to both traits.

Acknowledgments

We wish to warmly thank the patients for participating in this study. Teppo Varilo is greatly appreciated for family history investigation; Janet Sinsheimer and Tero Hiekkalinna are thanked for statistical advice; Jaana Hartiala, Susanna Vikman, Jennifer Schmidt, Ali Kuraishy, Maikki Parkkonen, Arja Tapio, and Tuula Airaksinen are thanked for technical assistance. This study was supported by the Finnish Cultural Foundation, Sigrid Jusélius Foundation, Finnish Heart Foundation, The Center of Excellence in Disease Genetics of the Academy of Finland, the Folkhälsan Research Foundation, The Helsinki University Central Hospital, the Finnish Diabetes Research Foundation, the EC (BM4-CT95-0662), the Swedish Medical Research Council, The Novo Nordisk Foundation, the Juvenile Diabetes Foundation – Wallenberg Foundation and Crafoord Foundation, Pahlssons Foundation and Swedish Research Council. This work was performed mainly at the National Public Health Institute in Helsinki under the research program of Molecular Medicine of Biomedicum and at the Department of Human Genetics, David Geffen School of Medicine at UCLA.

1. National Task Force on the Prevention and Treatment of Obesity. 2000. Overweight, obesity and health risk. *Arch. Intern. Med.* **160**:898–904.
2. Stunkard, A.J., Harris, J.R., Pedersen, N.L., and McClearn, G.E. 1990. The body-mass index of twins who have been reared apart. *N. Engl. J. Med.* **322**:1483–1487.
3. Moll, P.P., Burns, T.L., and Lauer, R.M. 1991. The genetic and environmental sources of body mass index variability: the Muscatine Ponderosity Family Study. *Am. J. Hum. Genet.* **49**:1243–1255.
4. Korkeila, M., Kaprio, J., Rissanen, A., and Koskenvuo, M. 1995. Consistency and change of body mass index and weight. A study on 5967 adult Finnish twin pairs. *Int. J. Obes. Relat. Metab. Disord.* **19**:310–317.
5. Echwald, S.M. 1999. Genetics of human obesity: lessons from mouse models and candidate genes. *J. Intern. Med.* **245**:653–666.
6. Chagnon, Y.C., et al. 2003. The human obesity gene map: the 2002 update. *Obes. Res.* **11**:313–367.
7. Iman, P.G. 2000. Obesity as a medical problem. *Nature.* **404**:635–643.
8. Lahti-Koski, M., Jousilahti, P., and Pietinen, P. 2001. Secular trends in body mass index by birth cohort in eastern Finland from 1972 to 1997. *Int. J. Obes. Relat. Metab. Disord.* **25**:727–734.
9. Rissanen, A., Heliövaara, M., Knekt, P., Reunanen, A., and Aromaa, A. 2003. Trends of obesity in Finland 1980–2000. *Int. J. Obes.* **27**:141. (Abstr.)
10. Peltonen, L. 2000. Positional cloning of disease genes: advantages of genetic isolates. *Hum. Hered.* **50**:66–75.
11. Öhman, M., et al. 2000. Genome-wide scan of obesity in Finnish sibpairs reveals linkage to chromosome Xq24. *J. Clin. Endocrinol. Metab.* **85**:3183–3190.
12. Wauters, M., and Van Gaal, L. 1999. Gender differences in leptin levels and physiology: a role for leptin in human reproduction. *J. Gen. Specif. Med.* **2**:46–51.
13. Blaak, E. 2001. Gender differences in fat metabolism. *Curr. Opin. Clin. Nutr. Metab. Care* **4**:499–502.
14. Harris, J.R., Tambs, K., and Magnus, P. 1995. Sex-specific effects for body mass index in the new Norwegian twin panel. *Genet. Epidemiol.* **12**:251–265.
15. Pietiläinen, K.H., et al. 1999. Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: a study of 4884 twins and 2509 singletons. *Int. J. Obes. Relat. Metab. Disord.* **23**:107–115.
16. Hager, J., et al. 1998. A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. *Nat. Genet.* **20**:304–308.
17. Stone, S., et al. 2002. A major predisposition locus for severe obesity, at 4p15-p14. *Am. J. Hum. Genet.* **70**:1459–1468.
18. Price, R.A., Li, W.D., and Kilker, R. 2002. An X-chromosome scan reveals a locus for fat distribution in chromosome region Xp21-22. *Diabetes* **51**:1989–1991.
19. Deng, H.W., et al. 2002. A genomewide linkage scan for quantitative-trait loci for obesity phenotypes. *Am. J. Hum. Genet.* **70**:1138–1151.
20. Almasy, L., and Blangero, J. 1998. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am. J. Hum. Genet.* **62**:1198–1211.
21. Monaghan, K.G., Van Dyke, D.L., and Feldman, G.L. 1998. Prader-Willi-like syndrome in a patient with an Xq23q25 duplication. *Am. J. Med. Genet.* **80**:227–231.
22. Pajukanta, P., et al. 1998. Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. *Nat. Genet.* **18**:369–373.
23. Soro, A., et al. 2002. Genome scans provide evidence for low-HDL-C loci on chromosomes 8q23, 16q24.1-24.2, and 20q13.11 in Finnish families. *Am. J. Hum. Genet.* **70**:1333–1340.
24. Vartiainen, E., et al. 2000. Cardiovascular risk factor changes in Finland 1972–1997. *Int. J. Epidemiol.* **29**:49–56.
25. Lindgren, C.M., et al. 2002. Genomewide search for type 2 diabetes mellitus susceptibility loci in Finnish families: the Botnia study. *Am. J. Hum. Genet.* **70**:509–516.
26. Parker, A., et al. 2001. A gene conferring susceptibility to type 2 diabetes in conjunction with obesity is located on chromosome 18p11. *Diabetes.* **50**:675–680.
27. Schwartz, S., et al. 2000. PipMaker—a web server for aligning two genomic DNA sequences. *Genome Research.* **10**:577–586.
28. Pielberg, G., Olsson, C., Syvänen, A.-C., and Andersson, L. 2002. Unexpectedly high allelic diversity at the *KIT* locus causing dominant white color in the domestic pig. *Genetics.* **160**:305–311.
29. Kruglyak, L., and Lander, E.S. 1995. Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am. J. Hum. Genet.* **57**:439–454.
30. Göring, H.H., and Terwilliger, J.D. 2000. Linkage analysis in the presence of errors III: marker loci and their map as nuisance parameters. *Am. J. Hum. Genet.* **66**:1298–1309.
31. Kruglyak, L., Daly, M.J., Reeve-Daly, M.P., and Lander, E.S. 1996. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am. J. Hum. Genet.* **58**:1347–1363.
32. Terwilliger, J.D. 1995. A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. *Am. J. Hum. Genet.* **56**:777–787.
33. Lange, K., et al. 2001. Mendel version 4.0: A complete package for the exact genetic analysis of discrete traits in pedigree and population data sets. *Am. J. Hum. Genet.* **69**:1886a.
34. Sloan, J.L., and Mager, S. 1999. Cloning and functional expression of a human Na⁺ and Cl⁻-dependent neutral and cationic amino acid transporter B⁰. *J. Biol. Chem.* **274**:23740–23745.
35. Benton, D. 2002. Carbohydrate ingestion, blood glucose and mood. *Neurosci. Biobehav. Rev.* **26**:293–308.
36. van der Mast, R.C., and Fekkes, D. 2000. Serotonin and amino acids: partners in delirium pathophysiology? *Semin. Clin. Neuropsychiatry.* **5**:125–131.
37. Roca, P., Proenza, A.M., and Palou, A. 1999. Sex differences in the effect of obesity on human plasma tryptophan/large neutral amino acid ratio. *Ann. Nutr. Metab.* **43**:145–151.
38. Lahti-Koski, M., Vartiainen, E., Männistö, S., and Pietinen, P. 2000. Age, education and occupation as determinants of trends in body mass index in Finland from 1982 to 1997. *Int. J. Obes. Relat. Metab. Disord.* **24**:1669–1676.
39. Rioux, J.D., et al. 2001. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat. Genet.* **29**:223–228.
40. Hugot, J.P., et al. 2001. Association of *NOD2* leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature.* **411**:599–603.
41. Pajukanta, P., et al. 2000. Two loci on chromosomes 2 and X for premature coronary heart disease identified in early- and late-settlement populations of Finland. *Am. J. Hum. Genet.* **67**:1481–1493.