

# Variance-Component Analysis of Obesity in Type 2 Diabetes Confirms Loci on Chromosomes 1q and 11q

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### Abstract

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To study genetic loci influencing obesity in nuclear families with type 2 diabetes, we performed a genome-wide screen with 325 microsatellite markers that had an average spacing of 11 cM and a mean heterozygosity of ~75% covering all 22 autosomes. Genotype data were obtained from 562 individuals from 178 families from the Breda Study Cohort. These families were determined to have at least two members with type 2 diabetes. As a measure of obesity, the BMI of each diabetes patient was determined. The genotypes were analyzed using variance components (VCs) analysis implemented in GENEHUNTER 2 to determine quantitative trait loci influencing BMI. The VC analysis revealed two genomic regions showing VC logarithm of odds (LOD) scores  $\geq 1.0$  on chromosome 1 and chromosome 11. The regions of interest on both chromosomes were further investigated by fine-mapping with additional markers, resulting in a VC LOD score of 1.5 on chromosome 1q and a VC LOD of 2.4 on chromosome 11q. The locus on chromosome 1 has been implicated previously in diabetes. The locus on chromosome 11 has been implicated previously in diabetes and obesity. Our study to determine linkage for BMI confirms the presence of quantitative trait loci influencing

obesity in subjects with type 2 diabetes on chromosomes 1q31-q42 and 11q14-q24.

**Key words:** type 2 diabetes, genome-wide screening, quantitative trait loci, genetics

The etiology of type 2 diabetes is ill defined: several studies indicate that the disease results from a combination of genetic susceptibility and external risk factors (1). According to this multifactorial model, genetically predisposed subjects will not necessarily develop overt disease unless they are also exposed to particular environmental factors (2). Important risk factors for the development of type 2 diabetes include a family history of diabetes, advanced age, hypertension, lack of physical exercise, and obesity (1).

Obesity, like diabetes, is a complex trait determined by multiple genetic and environmental factors (including physiological, behavioral, and sociocultural factors) (3). In recent years, several single-gene defects responsible for obesity in rodents, and also in humans in rare instances of extended families, have been identified. In addition to leptin (Online Mendelian Inheritance in Man no. 164160), which is the most notable example, numerous other proteins and neuropeptides have recently been found to participate in a complex network regulating food intake and energy expenditure (4). The relationship between type 2 diabetes and obesity seems complex, and it is unknown the extent to which both diseases can manifest independently of each other. However, it is unlikely that all forms of obesity associate with type 2 diabetes or vice versa. It is, therefore, likely that a possible direct link between the two will be limited to a subset of patients.

To identify loci influencing obesity in relation to diabetes, a quantitative trait loci (QTL)<sup>1</sup> analysis of BMI was

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<sup>1</sup> Nonstandard abbreviations: QTL, quantitative trait locus (quantitative trait loci); VC, variance component; LOD, logarithm of odds.

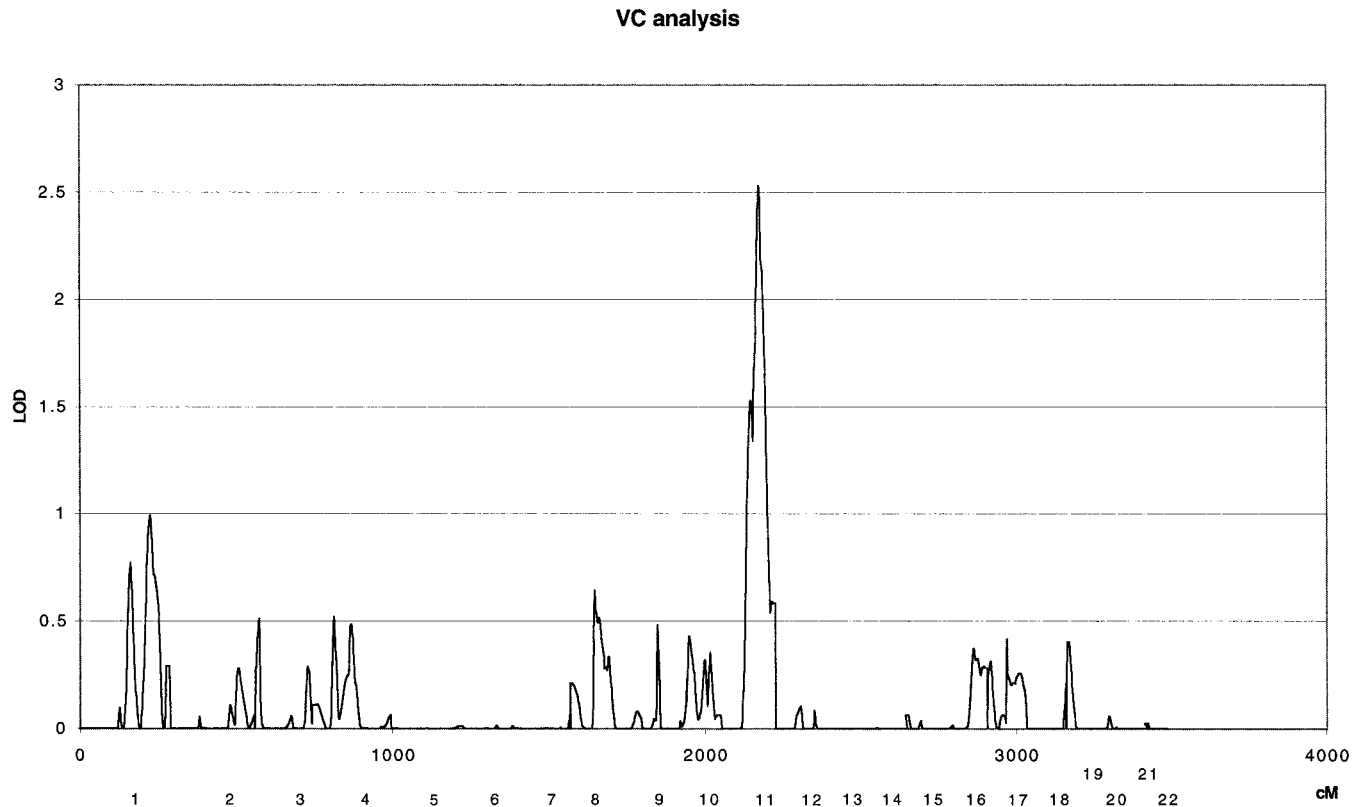


Figure 1: Multipoint VC analysis of BMI, with 325 autosomal microsatellite markers. Chromosome numbers on the  $x$  axis are placed at the midpoint of the respective chromosomes; length of chromosomes are adjusted according to the sex average map of the Marshfield genetic map (<http://research.marshfieldclinic.org/genetics>).

performed in subjects known to have type 2 diabetes. The variance-component (VC) analysis revealed two genomic regions showing VC logarithm of odds (LOD) scores  $\geq 1.0$  (see Figure 1). On chromosome 1, a VC LOD score of 1.0 was obtained between markers D1S1678 and D1S549, and, on chromosome 11, a VC LOD score of 2.5 was obtained between markers D11S940 and D11S2000. The regions of interest on both chromosomes were further investigated by fine mapping with additional markers.

The addition of four extra markers (D1S1660, D1S1663, D1S2141, and D1S179) on chromosome 1 increased the VC LOD from 1.0 to 1.5 between D1S1678 and D1S2141, whereas the addition of four extra markers (D11S1751, D11S3179, D11S1998, and D11S4464) on chromosome 11 slightly decreased the VC LOD from 2.5 to 2.3 between D11S1887 and D11S940 (see Figure 2).

Using a genome-wide screen of 562 individuals from 178 families who participated the Breda Study Cohort, we performed a QTL analysis for age- and gender-adjusted BMI. All individual family members were siblings; parents were not included in the study. These families had been asked to participate because at least two siblings were known to have type 2 diabetes; of the 562 participants, 420 were known to have type 2 diabetes.

The data were analyzed with multipoint VC analysis to find loci influencing BMI in diabetic subjects. However, any locus obtained may have also been involved with obesity in general. The results of the VC analysis suggest linkage of two obesity loci, one on chromosome 1q and one on chromosome 11q. Lander and Kruglyak (5) proposed that suggestive linkage was obtained at an LOD score of 2.2 ( $p = 0.001$ ) or higher; they defined significant linkage as an LOD score of 3.6 ( $p = 0.00002$ ) or higher. However, a typical 10-cM genome scan may fail to capture a significant part of the inheritance information, as occurs in multifactorial diseases, such as type 2 diabetes and obesity (6). We undertook dense mapping in regions of interest to circumvent this problem. For chromosome 1, we found a QTL that fell just short of suggestive linkage with a VC LOD score of 1.5, whereas the QTL at chromosome 11 showed suggestive evidence for linkage with a VC LOD score of 2.3.

The locus showing linkage to a QTL for BMI on chromosome 1 (LOD = 1.5; LOD-1 region 1q31-q42) has been found in various other linkage studies involving type 2 diabetes as a (sub)phenotype (6–8). Our findings and those of Wiltshire et al. (6), Meigs et al. (7), and Elbein et al. (8) focus particularly, at least in white subjects, on the region between markers D1S518 and D1S179. This region con-

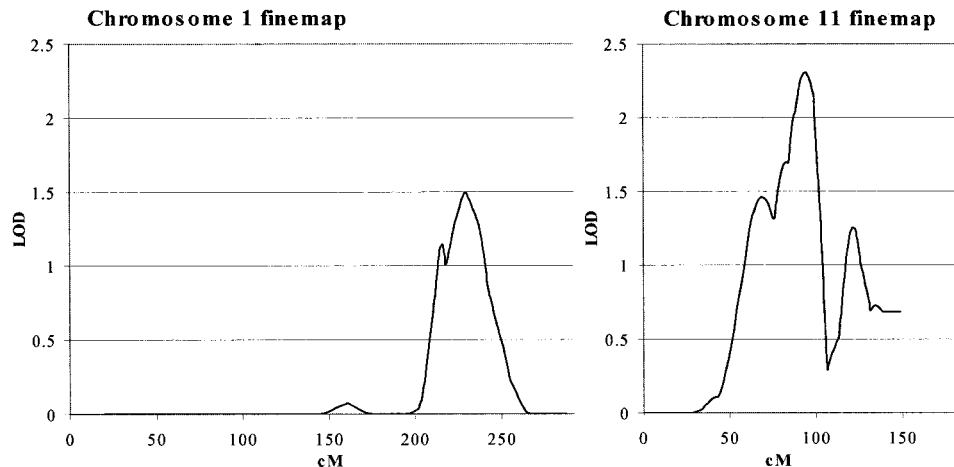


Figure 2: Fine-mapping results of chromosome 1 and 11. The solid line represents the VC linkage analysis of BMI.

tains the *CAPN2* and *CAPN9* genes, calcium-activated neutral proteases related to the *CAPN10* gene, which is a candidate gene associated recently with type 2 diabetes in a Mexican-American population (9). However, it is unlikely that these genes are involved in obesity and/or in type 2 diabetes because both are involved in different pathways and have been found to be associated with different diseases (10). Nevertheless, the similarity between our finding and these other studies supports the hypothesis that a diabetes-susceptibility locus may reside in this region on chromosome 1q and that it may well be that this locus acts under the influences of obesity. Thus far, no human QTL influencing obesity has been reported for this region on chromosome 1, although this region seems to be involved in different animal models for obesity and/or diabetes (11,12). The best animal model for this region is the TSOD (Tsumura, Suzuki, Obese, Diabetes) mouse, which is a model for obesity and diabetes. In this model, a QTL for body weight has been found on mouse chromosome region 1q25-q41, which shows synteny to human chromosome region 1q32-q41 (13).

The QTL found on chromosome 11 (LOD = 2.3; LOD-1 region 11q14-q24) in our analysis for BMI in the entire data set has also been shown previously to be involved in obesity and type 2 diabetes. Strong evidence for linkage has been found previously in a linkage study in Pima Indians (14). Nevertheless, to date, no physiological candidate genes have been found that account for linkage to obesity on chromosome region 11q23 (15). In spite of the findings of Hanson et al. (14), it remains unclear how excess accumulation of adipose tissue may increase the risk for diabetes. Obesity may cause diabetes by inducing insulin resistance in the liver and skeletal muscle (16) or by weakening  $\beta$ -cell function (17). This has been suggested to be mediated through disproportionate release of free fatty acids, tumor necrosis factor  $\alpha$ , other inflammatory cytokines, or energy-

related hormones by the adipocytes (18). As a consequence, obesity may represent an “environmental” risk factor for type 2 diabetes. In contrast, as stated above, obesity itself has a genetic predisposition, and some data suggest that specific loci linked to obesity traits may have pleiotropic effects. A gene influencing obesity could also influence insulin levels, as well as susceptibility to type 2 diabetes (14).

In summary, our findings support previous studies that have investigated loci on chromosomes 1q and 11q that most likely influence BMI. Because the subjects under investigation in our study had type 2 diabetes, these putative loci may also be involved in diabetes. Further studies in additional populations of obese patients, as well as in type 2 diabetic patients, will be necessary to provide a better insight into the complex interplay between obesity and type 2 diabetes.

## Research Methods and Procedures

### Population Studied

Selection and ascertainment of the Breda Study Cohort have been reported elsewhere (<http://humgen.med.uu.nl/research/diabetes/BredaCohort.html>). The study group comprised 562 individuals from 178 families (322 women and 240 men, 235 and 185 of whom, respectively, were diagnosed with type 2 diabetes). Families were included if at least two subjects taking part in the study were known to have type 2 diabetes. All participants from one family were siblings; no parents were studied. The level of obesity in each individual was determined according to BMI, defined as weight (kilograms) divided by height (meters) squared. The relationship between BMI and age and gender was determined by multiple linear regression analysis. The raw BMI values were adjusted in all family members for age and gender according to the obtained regression coefficients. For statistical analysis, logarithmic transformation of the

**Table 1.** Clinical characteristics of the study participants

Variables	Affected		Unaffected	
	Female	Male	Female	Male
Number ( <i>n</i> )	235	185	87	55
Age	69 ± 9	67 ± 9	64 ± 10	64 ± 10
Age at onset	58 ± 10	57 ± 9		
Body weight	73.9 ± 12.2	83.0 ± 12.6	72.3 ± 15.4	78.6 ± 8.5
BMI	27.9 ± 4.1	26.9 ± 3.4	26.4 ± 4.2	25.8 ± 2.2

Values are means ± SD.

BMI percentages of the subjects was performed to obtain a normal distribution. Table 1 shows the clinical information of the participants.

### Genotyping

A genome-wide screen was performed with 325 micro-satellite markers that had an average spacing of 11 cM and a mean heterozygosity of ~75% from 22 autosomes. For defining the map location of markers along the entire length of chromosomes, we used genetic map distances defined from the Marshfield genetic map (<http://research.marshfieldclinic.org/genetics>; see also the complementary marker information on the Web site of our own institution: <http://humgen.med.uu.nl/publications>). The markers were analyzed as described by van Tilburg et al. (19).

### Statistical Analyses

The resulting genotypes were analyzed using the multipoint VC method implemented in the software GENEHUNTER 2.0 (20–22), assuming an additive model and applying all-possible-sib-pairs (unweighted) analysis option. The VC method assumes that the expected genetic covariance between relatives for a trait is a function of the estimated proportion of alleles shared identically by descent at a linked marker locus. The identically-by-descent probabilities were estimated using a multipoint approach that considers all available genotypes. The likelihood-ratio test was applied to test the null hypothesis of no additive genetic variance attributable to a QTL at a particular location.

Allele frequencies were calculated from the entire data set. For analysis of the entire length of the different chromosomes, we used genetic map distances estimated from the Marshfield genetic map (<http://research.marshfieldclinic.org/genetics>; see also the complementary marker information on the Web site of our own institution: <http://humgen.med.uu.nl/publications>).

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