Genome-wide screen in obese pedigrees with type 2 diabetes mellitus from a defined Dutch population

J. H. van Tilburg, L. A. Sandkuijl, L. Franke, E. Strengman, P. L. Pearson, T. W. van Haeften and C. Wijmenga

University Medical Center, Utrecht, the Netherlands

Abstract

A genome scan was performed in obese type 2 diabetes mellitus pedigrees to identify susceptibility loci involved in obesity-driven type 2 diabetes mellitus. We studied the 20% most obese diabetes pedigrees from a confined Dutch population from around the town of Breda.

Previously we, and others, have already shown that a susceptibility locus influencing obesity in diabetes may reside on chromosome 18p11. We now report evidence to also suggest linkage for type 2 diabetes in these obese pedigrees on chromosome regions 11p (genome-wide P-value $\leq 0\cdot061$) and 12q (genome-wide P-value $\leq 0\cdot029$), thereby confirming previous findings from corresponding regions. The linkage found in the Breda Cohort of type 2 diabetes patients is influenced by obesity. This supports the notion that a genetic predisposition to obesity is probably intertwined with a genetic predisposition to type 2 diabetes. Further efforts should address the question of how, on a genetic level, these two factors interact.

Keywords Affected sibpairs, genomewide scan, obesity, type 2 diabetes.
Eur J Clin Invest 2003; 33 (12): 1070–1074

Introduction

The aetiology of type 2 diabetes is poorly 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, increased age, hypertension, lack of physical exercise, and obesity [1].

Several genome-wide scans for linkage with type 2 diabetes have been conducted over the past 5 years, and have

detected linkage with many genetic loci in various populations. This illustrates either the genetic heterogeneity of type 2 diabetes or the inability to replicate linkage with defined loci. However, at least one susceptibility gene, namely CAPN10, was found using a genome-wide scan approach [3].

Obesity is the greatest risk factor for type 2 diabetes mellitus, as it is known to induce insulin resistance via various mechanisms (TNFα release, free fatty acids, etc.). Both obesity and type 2 diabetes mellitus are complex traits determined by multiple genetic and environmental factors (including physiological, behavioural and sociocultural factors) [4]. In recent years, several single-gene defects responsible for obesity have been identified in rodents and also in humans in rare instances of extended families. In addition to leptin (OMIM:164160), which is the most notable example, numerous other proteins and neuropeptides have recently been found that participate in a complex network regulating food intake and energy expenditure [5]. The genetic relationship between type 2 diabetes and obesity appears complex and it is unknown how these two diseases influence each other at the genetic level. It seems unlikely that all forms of obesity will be associated with type 2 diabetes, or vice versa, and therefore any possible direct link between the two at the genetic level would probably be limited to a subset of patients.

To study further the relationship between type 2 diabetes and obesity we performed a genome-wide screen in a cohort of type 2 diabetes patients with known BMI values. The aim

of the study was to identify loci responsible for the restricted phenotype 'obesity-driven type 2 diabetes', using affected sibpair (ASP) analysis in obese diabetes patients. The ASP approach is well suited to the analysis of type 2 diabetes mellitus because only one or two generations in a family with this disease are normally available. The basis of the ASP analysis is that individuals concordant for a given genetic trait should show greater than expected concordance for marker alleles that are closely linked to the disease. In the absence of linkage, ASPs show on average a mean sharing in 50% of their alleles. However, if a marker is linked to a disease locus, ASPs show an allele sharing of more than 50%; this is called excess of allele sharing [6].

We stratified our cohort of 178 families with type 2 diabetes based on BMI values [7] and the 20% most obese type 2 diabetes pedigrees comprised 44 affected sibpairs with a mean BMI of 31.9 ± 2.6.

Materials and methods

Subjects

The Breda study cohort comprised 562 individuals from 178 families (322 women and 240 men, of whom 235 and 185, respectively, were diagnosed as having type 2 diabetes). The level of obesity in each individual was given by the body mass index (BMI), defined as weight (in kilograms) divided by height (in meters) squared. The relationship between BMI, age and gender was determined via multiple linear regression analysis. The raw BMI values were adjusted for age and gender in all family members according to the obtained regression coefficients, and normalized percentile values obtained following natural log transformation. Family members were classified as affected only if they had both type 2 diabetes and a high BMI level. As described previously [7] various datasets were made ranging from the 20% to 50% most obese pedigrees, comparable to Parker *et al.* [8] The subset analysis was performed on the 20% most obese pedigrees (the highest 20% of the adjusted BMI distribution within the study group). As a consequence the

20% most obese pedigrees' group contained only 44 affected sibpairs from 30 families (range adjusted BMI 28.5–43.5 kg m^{-1} [2]) (Table 1). Selection and ascertainment of the Breda Cohort has been reported elsewhere (http://humgen.med.uu.nl/research/diabetes/BredaCohort.html).

Genotyping

A modified version of the Weber set 6 containing 325 markers from 22 autosomes with at an average spacing of 11 cm was used for the genome-wide screen. Complementary marker information can be found at: http://humgen.med.uu.nl/publications/. The markers were analyzed as described by van Tilburg *et al.* [7].

Statistical analysis

Linkage analysis in type 2 diabetes patients with a high BMI was performed using MAPMAKER/SIBS software 2.0 package [9] and is described elsewhere [7]. Relationships between siblings were verified using the program GRR [10].

Permutation analysis

The genome-wide empirical significance of regions of excess allele sharing was determined by permutation analysis. In short, 10 000 replicates (incorporating the exact pattern of missing genotypes as observed in the genome scan) were generated from 44 affected sibpairs, randomly picked from the original dataset and analyzed by MAPMAKER/SIBS, as above.

Results

A whole genome scan using 325 markers with an average spacing of 11 cm was conducted. The affected sibpair analysis of these 44 sibpairs revealed four genomic regions

**Table 1** Distribution of variables used for subphenotypic classification according to BMI

	Whole data set (n = 562)		Affected (n = 420)		Highest percentile threshold values 20% most obese pedigrees
	Mean ± SD	Range	Mean ± SD	Range	
age (year)	67 ± 9	33–96	68 ± 9	41–96	65 ± 10
age at onset (year)	58 ± 10	35–85	57 ± 11	35–85	55 ± 10
Weight (kg)	77·5 ± 13·3	40–160	78·3 ± 13·3	40–128	90·3 ± 11·2*
BMI (kg m^{-1} [2])	27·2 ± 3·8	17·2–43·3	27·5 ± 3·9	17·1–43·3	31·9 ± 2·6†
No. of families	178		30		
No. of affected sibpairs	312		44		

*P-value < 3.4×10^{-12} (comparing both groups using Student's *t*-test).
†P-value < 3.4×10^{-12} (comparing both groups using Student's *t*-test).

LOD 20% most obese pedigrees
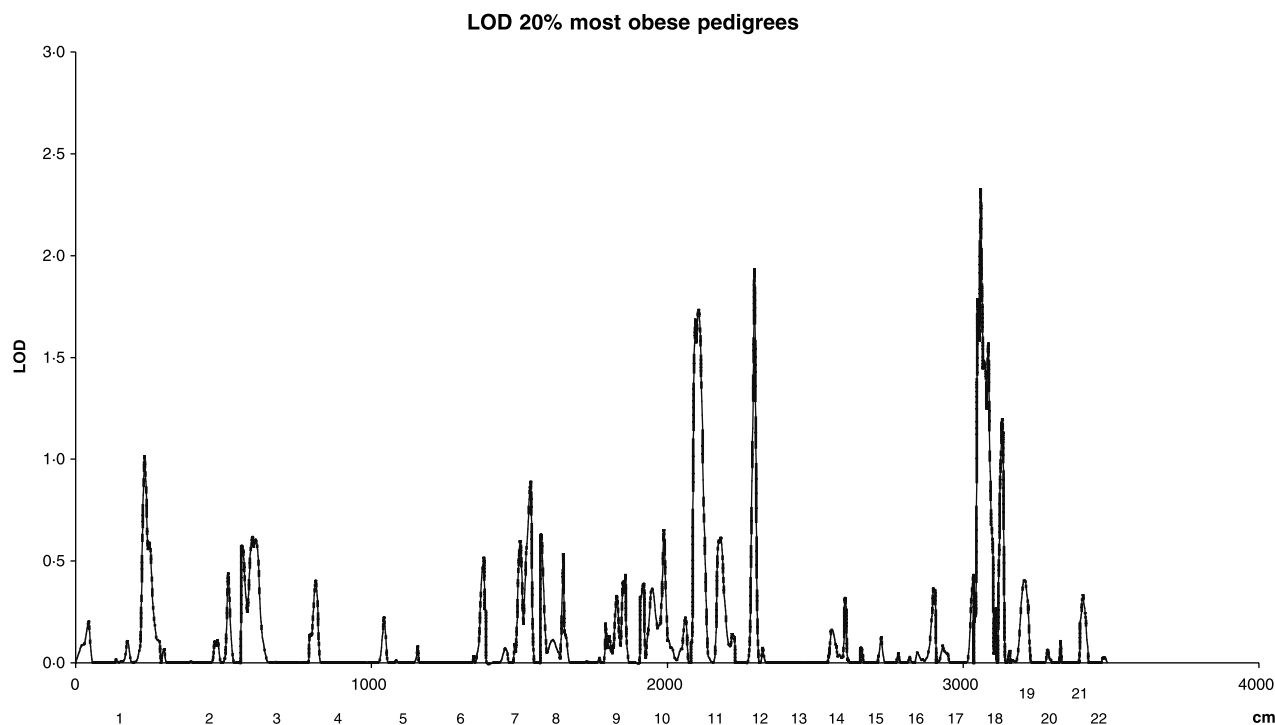
Figure 1 Multipoint nonparametric linkage analysis of the 20% most obese pedigrees, with 325 autosomal microsatellite markers. Chromosome numbers on the *X*-axis are placed at the midpoint of the respective chromosomes; length of chromosomes adjusted according to the sex average map of the Marshfield genetic map (http://research.marshfieldclinic.org/genetics/).
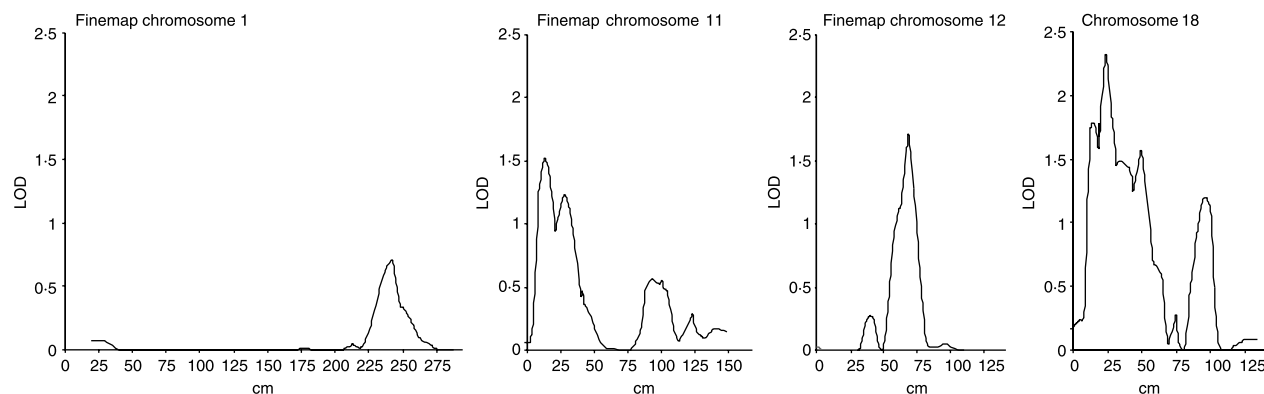
Figure 2 Fine-map results of chromosomes 1, 11, 12 and 18. Solid line represents the nonparametric linkage analysis of the 20% most obese type 2 diabetes families. LOD score chromosome 1q = 0·7 (genome-wide *P*-value ≤ 0·26); LOD score chromosome 11p15 = 1·5 (genome-wide *P*-value ≤ 0·061); LOD score chromosome 12q = 1·7 (genome-wide *P*-value ≤ 0·029); and LOD score chromosome 18p11 = 2·3 (genome wide *P*-value ≤ 0·028).

showing a maximum LOD scores of 1·0 (see Fig. 1). On chromosome 1 a maximum LOD score of 1·0 was found near marker D1S549. Addition of four extra markers in this region decreased the maximum LOD from 1·0 to 0·7 (genome-wide *P*-value ≤ 0·26). On chromosome 11, a maximum LOD score of 1·7 was obtained between markers D11S2362 and ATA34E08; addition of three extra markers between these markers slightly decreased the maximum LOD from 1·7 to 1·5 (genome-wide *P*-value ≤ 0·061)

between markers D11S2362 and D11S1999. For chromosome 12, a maximum LOD score of 1·9 was obtained near marker D12S1042; after adding six extra markers the maximum LOD of 1·9 decreased to a maximum LOD score of 1·7 (genome-wide *P*-value ≤ 0·029) between D12S1207 and D12S398 (see Fig. 2). We have previously indicated that a susceptibility locus influencing obesity in diabetes may reside on chromosome 18p11 (van Tilburg *et al.* [7]). For chromosome 18, a maximum LOD score of 2·3 was

obtained between markers D18S471 and D18S843 (genome-wide *P*-value ≤ 0·028). Chromosome 18 had already been fine-mapped to 5 cm or less, and no further genotyping was performed in this region.

Discussion

To study the relationship between type 2 diabetes and obesity we performed a genome-wide screen in a cohort of obese type 2 diabetes patients (mean BMI 31·9 ± 2·6). As reported previously [7,8] a linkage was ascribed to chromosome 18p11; two additional loci on 11p15 and 12q12-q14 were identified that may contain obesity-associated diabetes genes. To date, no plausible candidate genes have been proposed for the chromosome 18 location. The region between markers D11S2362 and D11S1999 on the short arm of chromosome 11 (LOD = 1·5; LOD-1 region 11p15) shows suggestive evidence for linkage to obese type 2 diabetes. This region harbours the insulin (INS) gene and its VNTR; it also harbours the sulphonylurea receptor 1 (SUR1) gene. Various association studies, although not conclusive, have found that the INS VNTR locus has been implicated in type 2 diabetes. More recent studies have established a significant association between the class III VNTR allele size and type 2 diabetes in Caucasian subjects in the UK [11,12]. Thus, insulin deficiency in type 2 diabetes might depend on polymorphisms in the VNTR, affecting the expression of the insulin gene. Sulphonylurea receptor 1 has been proposed as a candidate gene for type 2 diabetes, as it is a major determinant of normal glucose-induced insulin secretion in the beta-cell [13], and a target for the sulphonylurea type medication. It has been shown that an exon 18 variant of SUR1 was associated with morbid obesity and type 2 diabetes [14], although other sib-pair studies have failed to provide evidence for linkage in this region.

The region found on chromosome 12 (LOD = 1·7; LOD −1 region 12q12−q14) shows suggestive evidence for linkage in obese type 2 diabetes. This region has been shown to harbour the gene for vitamin D_3 receptor (VDR); allelic variations in VDR were reported to modulate insulin secretion in response to glucose [15]. It was also found that polymorphisms in the VDR gene were associated with the susceptibility to obesity in subjects with early onset type 2 diabetes [15].

Subsequently, other studies also showed evidence for linkage to chromosome 12q for type 2 diabetes [16,17]. This, with our data, suggests that a susceptibility locus for type 2 diabetes in combination with obesity resides on chromosome 12q. To date, no physiologically plausible candidate gene has been proposed that might account for the described linkage to diabetes on chromosome region 12q.

In summary, our study to determine linkage in obese type 2 diabetes families confirmed previous findings on various chromosomes (summarized in Table 2). We found evidence suggesting linkage for type 2 diabetes on chromosome regions 11p and 12q, apart from the previously reported locus on 18p11 [7,8], which confirm previous findings to

Table 2 Summary of results

Locus	LOD	Previously described in the literature
11p	1·5	Implicated in candidate gene analysis for T2D [11,12,14]
12q	1·7	Linkage studies in T2D [16,17]
18p11 [7]	2·3	Linkage study in T2D [8]

T2D, type 2 diabetes.

the corresponding regions. The linkage determined in the Breda cohort of type 2 diabetes patients is influenced by obesity. Previous studies did not consider the role of BMI, with the exception of the 18p11 locus, where a similar BMI stratification was applied [8].

From the present data it cannot be inferred whether individual BMI loci are independently or cooperatively involved in determining diabetes status. Further studies in other populations of obese patients, as well as in type 2 diabetes patients, will be necessary to provide better insight into the interplay between loci and/or genes primarily associated with obesity and those primarily associated with type 2 diabetes. Ideally, two independent groups from the same population should be studied, one ascertained for obesity independent of diabetes status and, vice versa, one ascertained for type 2 diabetes independently of BMI.

References

1 DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991;**14**:173–94.
2 Valsania P, Micossi P. Genetic epidemiology of non-insulin-dependent diabetes. *Diabetes Metab Rev* 1994;**10**:385–405.

3 Horikawa Y, Oda N, Cox NJL, Li X, Orho-Melander M, Hara M *et al.* Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 2000;**26**:163–75.

4 Perusse L, Bouchard C. Genotype–environment interaction in human obesity. *Nutr Rev* 1999;**57**:S31–7; S37–8.

5 Rankinen T, Perusse L, Weisnagel SJ, Snyder EE, Chagnon YC, Bouchard C. The human obesity gene map: the. *Update Obes Res* 2001;**10**:196–243. 2002.

6 Van Tilburg J, van Haeften TW, Pearson P, Wijmenga C. Defining the genetic contribution of type 2 diabetes mellitus. *J Med Genet* 2001;**38**:569–78.

7 van Tilburg JHO, Sandkuijl LA, Strengman E, van Someren H, Rigters-Aris T, Pearson PL *et al.* A genome-wide scan in type 2 diabetes mellitus provides independent replication of a susceptibility locus on 18p11 and suggests the existence of novel loci on 2q12 and 19q13. *J Clin Endocrinol Metab* 2003;**88**:2223–30.

8 Parker A, Meyer J, Lewitzky S, Rennich G, Chan JD, Thomas M *et al.* A gene conferring susceptibility to type 2 diabetes in conjunction with obesity is located on chromosome 18p11. *Diabetes* 2001;**50**:675–80.

9 Kruglyak L, Lander ES. Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am J Hum Genet* 1995;**57**:439–54.

10 Abecasis GR, Cherny SS, Cookson WO, Lardon LR *et al.* GRR: graphical representation of relationship errors. *Bioinformatics* 2001;**17**:742–3.

11 Huxtable SJ, Saker PJ, Haddad L, Walker M, Frayling TM, Levy JO *et al.* Analysis of parent-offspring trios provides evidence for linkage and association between the insulin gene and type 2 diabetes mediated exclusively through paternally transmitted class III variable number tandem repeat alleles. *Diabetes* 2000;**49**:126–30.

12 Ong KK, Phillips DI, Fall C, Poulton J, Bennet ST, Golding J *et al.* The insulin gene VNTR, type 2 diabetes and birth weight. *Nat Genet* 1999;**21**:262–3.

13 t Hart LM, Dekker JM, van Haeften TW, Ruige JB, Stehouwer CD, Erkelens DW *et al.* Reduced second phase insulin secretion in carriers of a sulphonylurea receptor gene variant associating with Type II diabetes mellitus. *Diabetologia* 2000;**43**:515–9.

14 Hani EH, Clement K, Velho G, Vionnet N, Hager J, Philippi A *et al.* Genetic studies of the sulfonylurea receptor gene locus in NIDDM and in morbid obesity among French Caucasians. *Diabetes* 1997;**46**:688–94.

15 YeWZ, Reis AF, Dubois-Laforgue D, Bellanne-Chantelot C, Timsit J, Velho G. Vitamin D receptor gene polymorphisms are associated with obesity in type 2 diabetic subjects with early age of onset. *Eur J Endocrinol* 2001;**145**:181–6.

16 Bektas A, Suprenant ME, Wogan LT, Plengvidhya N, Rich S, Warram JH *et al.* Evidence of a novel type 2 diabetes locus 50 cm centromeric to NIDDM2 on chromosome 12q. *Diabetes* 1999;**48**:2246–51.

17 Mahtani MM, Widen E, Lehto M, Thomas J, McCarthy M, Brayer J *et al.* Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. *Nat Genet* 1996;**14**:90–4.