

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

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A genome-wide scan was performed, using nonparametric linkage analyses, to find susceptibility loci for type 2 diabetes mellitus in the Dutch population. We studied 178 families from The Netherlands, who constituted 312 affected sibling pairs. The first stage of the genome scan consisted of 270 DNA markers, with an average intermarker spacing of 13 cM. Because obesity and type 2 diabetes mellitus are interrelated, the data set was stratified for the subphenotype body mass index, corrected for age and gender. This resulted in a suggestive maximum multipoint LOD score of 2.3 (single-point *P* value, 9.7×10^{-4} ; genome-wide *P* value, 0.028) for the most obese 20% ped-

igrees of the data set, between marker loci D18S471 and D18S843. In the lowest 80% obese pedigrees, two interesting loci on chromosome 2 and 19 were found, with LOD scores of 1.5 and 1.3. We provide independent evidence that the chromosome 18p11 locus, reported earlier from a Finnish/Swedish population, is of definite interest for type 2 diabetes mellitus in connection with obesity. Subsequently, our results indicate that two novel loci may reside on chromosomes 2 and 19, with minor effects involved in the development of type 2 diabetes mellitus in the Dutch population. (*J Clin Endocrinol Metab* 88: 2223–2230, 2003)

THE ETIOLOGY OF type 2 diabetes mellitus is unknown, but 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 mellitus, apart from obesity, include a family history of diabetes, increased age, hypertension, lack of physical exercise, and ethnic background (1).

The discovery of monogenic forms of diabetes, such as maturity-onset diabetes of the young, underscores the phenotypic and genotypic heterogeneity of this disease. Despite identification of at least six maturity-onset diabetes of the young loci to date, they account for only a few percent of all diabetes patients (3). However, defining the genetic basis of the far more common polygenic forms of type 2 diabetes mellitus presents methodological difficulties because of the absence of extended families and the small contribution of each polygene to the disease phenotype. Candidate gene studies have identified several loci with a modest effect on type 2 diabetes mellitus susceptibility (4). Several genome-wide scans have been conducted in the past 5 yr, giving rise to various genetic regions in as many various populations, illustrating the genetic heterogeneity of type 2 diabetes mellitus (5–18). However, at least one susceptibility gene was found using the genome-wide scan approach (CAPN10) (19). It is therefore of great importance that replication studies are performed in additional populations, as well as independent

genome scans in different populations, to confirm the original findings or to provide more insight into the genetic complexity of type 2 diabetes mellitus.

We describe a genome-wide linkage analysis to identify type 2 diabetes mellitus susceptibility loci in nuclear families from the province of North Brabant, The Netherlands (around the town of Breda). To minimize genetic heterogeneity, all nuclear families with at least two affected siblings were ascertained from a region of 130,000 inhabitants.

Subjects and Methods

Population studied

Probands were recruited in collaboration with their general practitioners and the Diabetes Service Breda, which is the only regional clinical and laboratory service for the western part of the province of North Brabant in The Netherlands. Since 1990, the Diabetes Service Breda has collected clinical and biochemical data on more than 13,000 patients with type 2 diabetes mellitus. All patients undergo clinical and laboratory evaluations for their diabetes at regular 3-month intervals. Initially, 4,000 possible participants were asked, at random, to take part in this study. Although we had a response of 60%, only those who had at least one affected sibling and were diagnosed after the age of 35 yr, according to World Health Organization criteria, were included. Unfortunately, no parents were available. If both parents were reported to have type 2 diabetes, the families were not included in this study. However, if available, unaffected relatives were included to reconstruct parental genotypes. Initially, 214 families fulfilled the criteria for inclusion in the study. After careful evaluation of the affected siblings, 36 families were excluded because one of the affected siblings was younger than 35 at age of onset of type 2 diabetes mellitus or because of demonstrable nonpaternity. The remaining 178 families, consisting of 420 patients and 142 unaffected siblings, were included in the genome-wide screen. These 178 families comprised 312 affected sibling pairs, with an average sibship size of 3.1; there were 128 families with two affected siblings, 40 families

Abbreviation: BMI, Body mass index; DS, diabetes subjects.

with 3 affected siblings, 9 families with 4 affected siblings, and 1 family with 5 affected siblings. The Medical Ethics Committee of the University Medical Center in Utrecht approved our study protocol, and all the participants signed an informed consent. The participants' clinical parameters are shown in Table 1.

Genotyping

DNA was extracted from 10 ml blood, using standard procedures (20). The 562 DNA samples were divided into eight 96-well microtiter plates. Every DNA plate contained up to 80 unique DNA samples, 6 blind duplicate samples, 3 Centre d'Etudes de Polymorphisme Humaine controls, and 1 negative control. A modified version of the Weber set 6, containing 270 markers (73% of markers from the Weber 6 map) at an average spacing of 13 cm, was used for the genome-wide screen. For details of the markers see: <http://humgen.med.uu.nl/publications/>. Reverse primers were labeled with either 6-FAM, HEX, or TET fluorescent dyes (Isogen Bioscience, Maarssen, The Netherlands) at the 5'-end. PCR was carried out in a 10- μ l volume containing 1 \times PCR Gold buffer (PE Applied Biosystems, Foster City, CA), 200 μ M deoxy-nucleoside triphosphate, 2.5 mM MgCl₂, 25 ng/ml of each primer, 0.4 U AmpliTaq Gold (PE Applied Biosystems), and 25 ng genomic DNA. Cycling conditions were: 7 min at 94 C, followed by 32 cycles of 30 sec at 95 C, 30 sec at 55 C, and 30 sec at 72 C, followed by a final extension at 72 C for 30 min. PCR products were pooled into 4 different running sets, before electrophoresis on an ABI 3700 (PE Applied Biosystems), and analyzed using GeneScan version 3.1 (PE Applied Biosystems). Allele sizes of the individual markers were determined using Genotyper version 2.1 software (PE Applied Biosystems). Centre d'Etudes de Polymorphisme Humaine reference samples (1331-01, 1331-02, and 1347-02) were included to determine the appropriate size of the alleles. The 48 duplicate samples were included to estimate the proportion of mistyping of genotypes. All samples were double-checked by two independent investigators (J.H.O.v.T. and E.S.), who did not know the origin of the 48 duplicate samples. The blind genotypes of the duplicate samples were compared with the original samples by a technician (Alfons Bardoel).

Stratification of the data set

Stratification was performed for a correct comparison with the data obtained by Parker *et al.* (15). In unaffected individuals ($n = 150$), the relationship between body mass index (BMI) and age and gender was determined via multiple linear regressions. For all family members, BMI was adjusted for age and gender according to the resulting regression coefficient. Normal percentile values were obtained from the adjusted BMI values in unaffected individuals. All affected individuals were then classified according to these percentiles. Only sibling pairs in which both affected siblings fit in the same percentile group were included in the stratified data set. Various data sets were made, ranging from 20–50% most obese pedigrees, comparable with Parker *et al.* (15). The subset analysis was performed on the 20% most obese pedigrees.

As a consequence, the 20% most obese pedigrees group [referred to as diabetes subjects (DS)-20%] contained only 44 affected sibling pairs from 30 families. As a counterpart to the DS-20% group, there was the group without the 20% most obese pedigrees (referred to as DS-80%); only sibling pairs who fit in this group were included, giving 146 affected sibling pairs (see Table 3).

TABLE 1. Clinical information of participants

	Affected		Unaffected	
	Female	Male	Female	Male
Number	235	185	87	55
Age	69 \pm 9	67 \pm 9	64 \pm 10	64 \pm 10
Age at onset	58 \pm 10	57 \pm 9		
Body weight	73.9 \pm 12.2	83.0 \pm 12.6	72.3 \pm 15.4	78.6 \pm 8.5
BMI	27.9 \pm 4.1	26.9 \pm 3.4	26.4 \pm 4.2	25.8 \pm 2.2
Affected sibling pairs				
All possible	312			
Independent	239			

Statistical analyses

To assess for linkage, we applied multipoint nonparametric linkage analysis, using the MapMaker/Sibs software 2.0 package (21). Allele frequencies were calculated from the whole data set, and the weighted sibling pair option in MapMaker/Sibs was used. Unaffected siblings were incorporated into the analysis for extra identity-by-descent information, and their status was set as unaffected. For analysis of the entire length of the different chromosomes, we used genetic map distances estimated from the Marshfield genetic map (<http://research.marshfield-clinic.org/genetics/>), see also the complementary information on the web site: <http://humgen.med.uu.nl/publications/>. Exclusion mapping was performed using the exclude option of MapMaker/Sibs, under an additive model and at several locus-specific values of λ_s (the ratio of the risk to sibling of an affected person relative to the risk to a member of the general population), ranging from 1.25–2.5. LOD scores for exclusion of a region were obtained by comparing the likelihood of the data, assuming the presence of a locus with a specific effect (λ_s), to the likelihood if the region containing no relevant locus at all ($\lambda_s = 1$).

Power calculations

Computer simulations were carried out on an initial marker map with a 20 cM spacing, with subsequent markers at 2-cm intervals, 250 sibling pairs, and marker information more than 0.7. This simulation showed that, with the initial marker map, we could only extract about 55% of all the available information from the sibling pairs. After saturation with additional markers [on average, 94% of the maximal information (which would be for a completely linked and completely informative marker) could be extracted via the MapMaker/Sibs program. Under the assumption of a single disease-predisposing locus for type 2 diabetes mellitus (locus-specific $\lambda_s = 3.5$), there was approximately 100% power for detection, with a LOD score of 3 (P value ≤ 0.0001), when the initial map of 270 markers was used. The power to detect a locus with a modest effect (locus-specific $\lambda_s = 1.5$) is approximately 70%. It should be realized, however, that if there are multiple disease-predisposing loci, each will present an opportunity for mapping. Therefore, whereas our sample size yields a power of 70% to detect a single locus with λ_s of 1.5, if there are 2 such loci, our power to detect at least one of them is $0.70 + [0.70 \times (1 - 0.70)] = 0.91$. Because type 2 diabetes mellitus is expected to be genetically heterogeneous, we were aiming at a LOD score of 0.5 ($P \leq 0.09$) in our initial scan. To detect a locus with a relatively small effect ($\lambda_s = 1.5$), the power is approximately 99% for detection, with a LOD score of 0.5.

Results

The autosomal genome scan was completed on 178 families consisting of 420 patients and 142 unaffected siblings, which were used to generate the final data set for statistical analysis. By including additional siblings, both affected and unaffected, we obtained an average sibship size of 3.1. The addition of unaffected siblings provided extra identity-by-descent information. The first stage of the genome scan consisted of 270 DNA markers, with an average intermarker spacing of 13 cm and a mean heterozygosity of 0.76. Forty-eight duplicate samples were included, to estimate the proportion of typing errors. An average of 90% of subjects was successfully genotyped for each marker, with less than 3% of mistyping of genotypes. The average information content was 50% throughout the genome [measuring the proportion of the total inheritance information extracted at each chromosomal position, given the observed genotype data (22)]; see <http://humgen.med.uu.nl/publications/> for details.

Because type 2 diabetes mellitus is expected to be genetically heterogeneous, we were aiming at a LOD score of 0.5 ($P \leq 0.09$) in our initial scan. Only four genomic regions initially showed multipoint LOD scores of 0.5 or more (Fig. 1). On chromosome 2, a LOD score of 0.5 was obtained

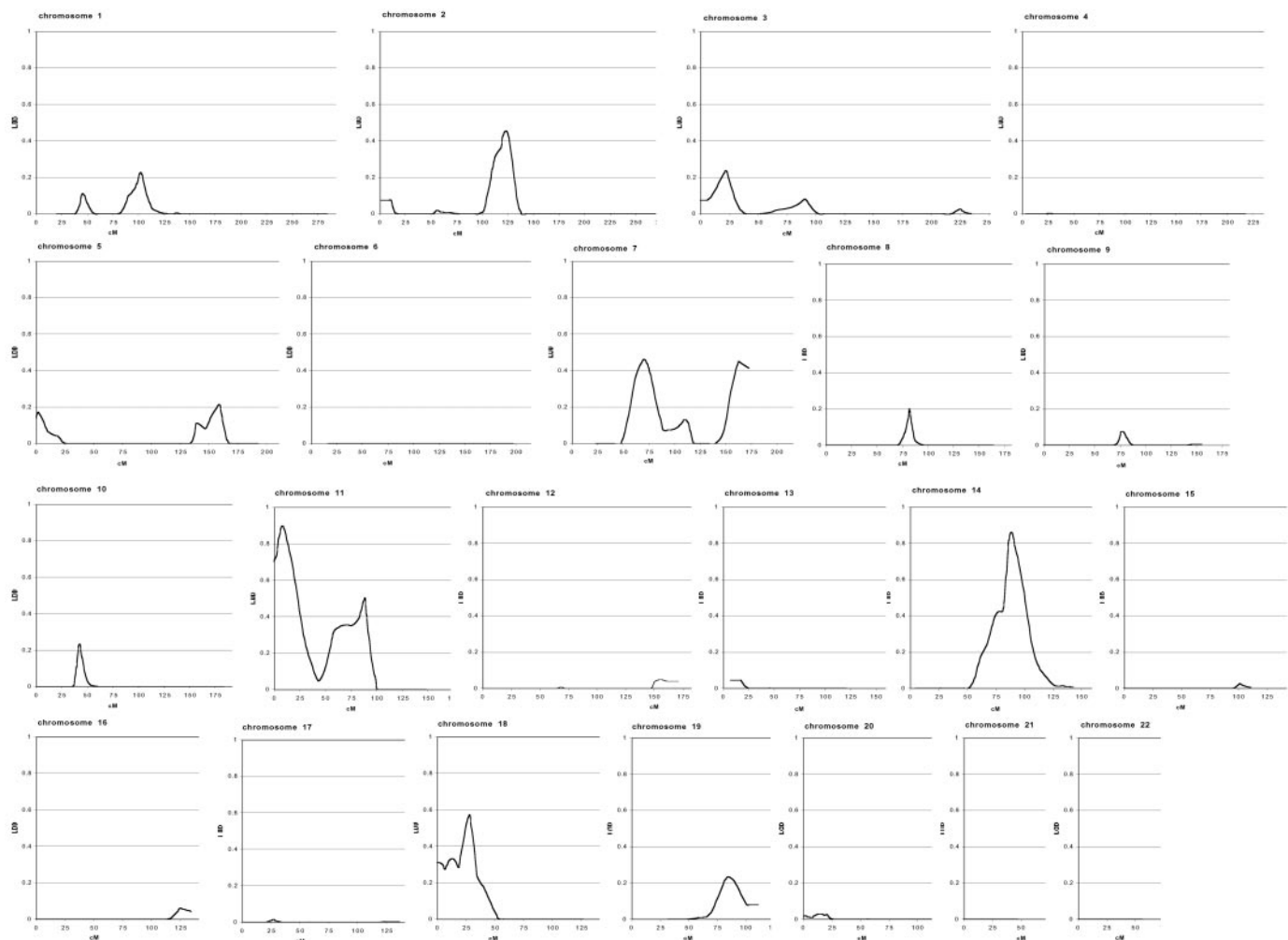


FIG. 1. Graphs of the multipoint LOD scores for each autosome from the genome-wide scan in 178 affected sibling pairs with 270 DNA markers, using the nonparametric linkage analysis program, MapMaker/Sibs. The x-axis represents the length of the chromosome in centimorgans, whereas the y-axis represents the multipoint LOD score. Both axes are presented on the same scale.

between markers D2S436 and D2S410; on chromosome 11, a maximum LOD score of 0.9 was obtained between markers D11S1984 and D11S2362; chromosome 14 showed a LOD score of 0.9 between markers D14S53 and D14S606; whereas on chromosome 18, a LOD score of 0.6 was obtained near marker D18S843. These four chromosomal regions were selected for follow-up studies (Table 2).

Fine maps of the regions exhibiting LOD scores of 0.5 or more were constructed, with an average intermarker spacing of 5 cM or less. The original 178 type 2 diabetes mellitus families were then genotyped using these fine maps. The results of the follow-up studies are summarized in Table 2. The addition of extra markers (10, 3, 6, and 15 markers for chromosomes 2, 11, 14, and 18, respectively) gave the following results: the multipoint LOD at chromosome 14 decreased from 0.9 to 0.3 at marker position D14S53, whereas the multipoint LOD on chromosome 11 decreased slightly from 0.9 to 0.8. No change in the multipoint LOD for chromosome 2 was seen after addition of extra markers. However, on chromosome 18, the multipoint LOD increased slightly when more markers were analyzed in the region of interest. Analyzing more markers on chromosome 18 increased the multipoint LOD from 0.6 at marker position

D18S843 to a multipoint LOD of 0.7 between markers D18S1163 and D18S843 (Table 2). For additional information on the markers, see <http://humgen.med.uu.nl/publications/>.

In addition to searching for evidence of linkage, we performed exclusion mapping to determine which genomic regions could be excluded as candidates for harboring major susceptibility loci. Five different locus-specific values of λ_s were considered (1.25, 1.5, 1.75, 2.0, and 2.5). We could exclude 87% of the genome for a λ_s of 2.0 (for details, see <http://humgen.med.uu.nl/publications/>). However, for a λ_s of 1.25, we could only exclude 7% of the genome with a sample of 312 affected sibling pairs and in the absence of parental data. This is almost certainly attributable to the limited size of our data set in resolving such low predisposition values, suggesting that large numbers of affected sibling pairs are necessary to identify loci with minor effects in type 2 diabetes mellitus.

Stratification for obesity

A previous study by Parker *et al.* (15) had reported a locus on chromosome 18 whose genetic contribution increased by

TABLE 2. Regions displaying multipoint LOD scores of 0.5 or more in Dutch Caucasian siblings with type 2 diabetes mellitus

Chromosome	Marker	cM	Het	LOD	<i>P</i> value ^a	
Dense map ^b	2	D2S436	118.2	0.90	0.3	0.16
		Interval	123.7		0.5	0.09
		D2S410	125.2	0.81	0.3	0.16
	11	D11S1984	2.1	0.76	0.7	0.04
		Interval	7.6		0.9	0.03
		D11S2362	8.9	0.93	0.9	0.04
	14	D14S53	86.3	0.67	0.8	0.12
		Interval	88.5		0.9	0.03
		D14S606	91.6	0.69	0.8	0.05
	18	D18S843	28.1		0.6	0.07
	2	D2S436	118.2	0.90	0.0	0.58
		D2S1888	121.6	0.71	0.2	0.22
		Interval	122.9		0.5	0.09
		D2S410	125.2	0.81	0.4	0.12
	11	D11S2362	8.9	0.93	0.8	0.04
	Interval	10.2		0.8	0.04	
	D11S1999	17.9	0.76	0.7	0.05	
14	D14S53	86.3	0.67	0.3	0.16	
	Interval	88.5		0.3	0.16	
	D14S606	91.6	0.69	0.1	0.22	
18	D18S1163	24.1	0.54	0.7	0.05	
	Interval	27.2		0.7	0.05	
	D18S843	28.1		0.7	0.05	
Stratification ^c						
DS-20%	D18S452	18.7	0.83	2.3	9.5×10^{-4}	
	Interval	24.0		2.5	5.5×10^{-4}	
DS-20% ^d	D18S1163	24.1	0.54	2.4	7.1×10^{-4}	
	D18S1163	24.1		2.3	0.0011	
	Interval	24.8		2.3	9.7×10^{-4}	
	D18S843	28.1		1.8	0.003	

Het, Heterozygosity of the marker.

^a Single-point *P* value according to Holmans' (39) possible triangle method.^b With the addition of more markers at a spacing of at least 5 cM in the region of interest.^c Analysis using data set stratified on age- and sex-adjusted BMI.^d With the addition of five extra markers in the region between D18S452 and D18S1163 on chromosome 18.**TABLE 3.** Distribution of variables used for subphenotypic classification according to BMI

	Whole data set		Affected		Highest percentile threshold values	
	Mean \pm SD	Range	Mean \pm SD	Range	DS-20%	DS-80%
Age (yr)	67 \pm 9	33–96	68 \pm 9	41–96	65 \pm 10	68 \pm 9
Age at onset (yr)	58 \pm 10	35–85	57 \pm 11	35–85	55 \pm 10	58 \pm 10
Weight (kg)	77.5 \pm 13.3	40–160	78.3 \pm 13.3	40–128	90.3 \pm 11.2	72.4 \pm 11.3
BMI (kg/m ²)	27.2 \pm 3.8	17.2–43.3	27.5 \pm 3.9	17.1–43.3	31.9 \pm 2.6	25.3 \pm 2.4
No. of families		178			30	99
No. of affected sibling pairs		312			44	146

stratifying the data set for BMI. When a comparable stratification was applied to our data set (Table 3), the multipoint LOD increased from 0.7 in the unstratified sample to LOD 2.5 (between markers D18S452 and D18S1163) when the DS-20% pedigrees were analyzed (Table 2). We saw a clear decline in the DS-30%, DS-40%, and DS-50% (data not shown), giving the highest LOD in the DS-20%. These data suggest the actual cut-off value is expected to be around the 20th percentile. Adding another eight markers to this region, with a resolution of one marker every 1.5 cM, slightly decreased the LOD, to a maximum LOD of 2.3 for the DS-20% pedigrees, between markers D18S471 and D18S843 near marker D18S1163 (Table 2).

Figure 2 illustrates the evident effect of BMI stratification on the multipoint LOD score, despite the reduction in sample size. Only 30 families, containing 44 affected diabetes sibling pairs, comprised the DS-20% type 2 diabetes mellitus pedigrees. The mean BMI of the affected individuals in this subphenotype group of DS-20% pedigrees was 31.9 kg/m² *vs.* 27.5 kg/m² for all affected individuals in the whole data set. Although the overall marker information content was relatively low, namely 50% (see <http://humgen.med.uu.nl/publications/>), it did reach 78% at the map position showing the highest evidence for linkage on 18p, with an average information content of 91% between D18S391 and D18S1163.

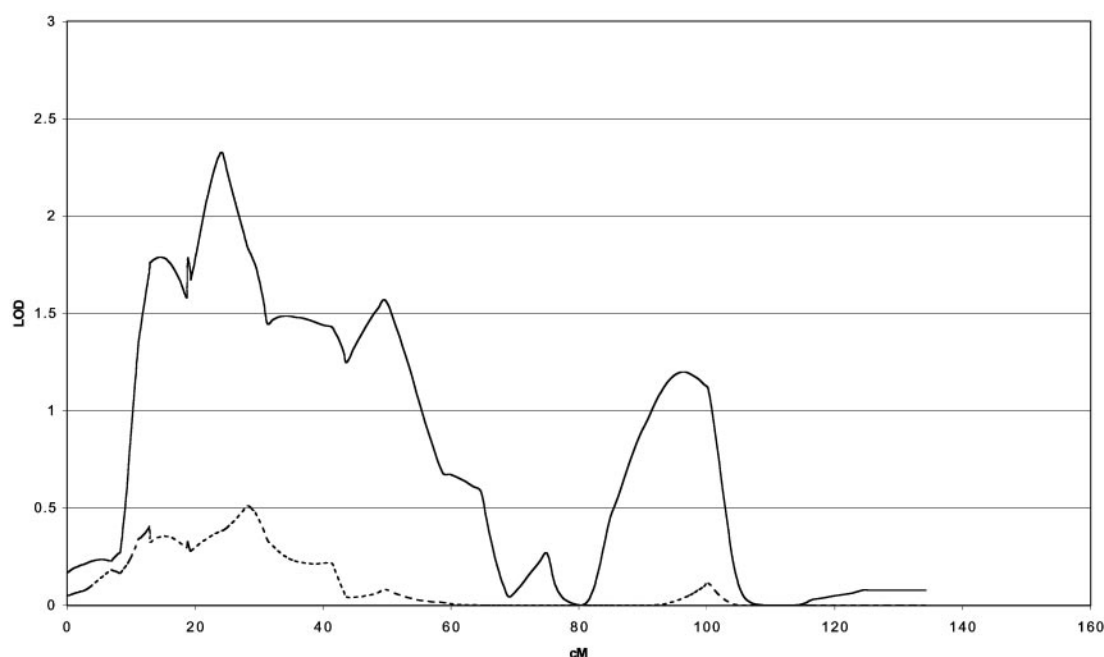


FIG. 2. Fine-map (33 markers) analysis of chromosome 18 in a data set stratified by mean age- and sex-adjusted BMI. The *dashed line* indicates the whole data set; and the *solid line*, the DS-20% set. The confidence limits define this locus as lying between D18S967 and D18S1153, a region of approximately 20 cm.

TABLE 4. Regions displaying multipoint LOD scores of at least 1.0 in 146 DS-80% sibpairs

Chromosome	Marker	cM	Het	LOD	<i>P</i> value ^a
2 ^b	D2S436	118.2	0.90	1.0	0.0239
	Interval	121.4		1.5	0.0075
15	D2S1888	121.6	0.71	1.4	0.0092
	D15S817	4.78	0.79	0.8	0.0446
	Interval	20.28		3.1	1.54×10^{-4}
19	ACTC	31.5	0.94	0.8	0.0423
	D19S400	64.7	0.86	0.6	0.0683
	Interval	79.5		1.5	0.0063
	D19S245	100.6	0.76	0.3	0.1600
Dense map					
15	ACTC	31.5	0.94	0.3	0.1802
19	D19S246	78.1	0.84	1.0	0.0239
	Interval	80.0		1.3	0.0112
	D19S601	83.2	0.81	1.2	0.0144

Het, Heterozygosity of the marker.

^a Single point *P* value according to Holmans' (39) possible triangle method.

^b This is already a dense map with markers at 5 cm or less.

Genome screen in DS-80%

The limited number of interesting loci resulting from the genome-wide scan prompted us to recalculate the LOD scores for the affected sibling pairs in which both siblings were in the DS-80% group, because we thought this group could represent a different subphenotype. The DS-80% consisted of 146 affected sibling pairs from 99 families, with 220 patients and 72 unaffected siblings, with an average sibship size of 2.9 (Table 3). The number of markers was increased from 270 to 319, because markers were added to fine-map the 4 regions already identified. Recalculation of the LOD scores was performed for 319 markers; interestingly, 3 genomic regions showed multipoint LOD scores of 1.0 or more (Table 4). On chromosome 2, a LOD score of 1.5 was obtained

between markers D2S436 and D2S1888, a region also identified in the whole data set. Because this region had already been saturated with markers every 5 cM or less, we did not perform further fine-mapping on this region. On chromosome 15, a maximum LOD score of 3.1 was obtained between markers D15S817 and the gene ACTC; whereas on chromosome 19, a LOD score of 1.5 was obtained between markers D19S400 and D19S254. All results of the recalculation can be seen on <http://humgen.med.uu.nl/publications/>.

Both regions on chromosomes 15 and 19 were further investigated by fine-mapping. Addition of four markers on chromosome 15 showed that the previous finding was a false positive finding, because the maximum LOD score decreased from 3.1 to 0.3 near the ACTC gene. However, adding three

markers to the map of chromosome 19 gave the following result: the maximum LOD score on chromosome 19 decreased slightly, from 1.5 to 1.3, between markers D19S246 and D19S601 (Table 4).

Discussion

We performed a genome-wide linkage analysis study designed to identify type 2 diabetes mellitus susceptibility loci in type 2 diabetes mellitus nuclear families from Breda, North Brabant, The Netherlands. Our results contribute to a better understanding of type 2 diabetes mellitus, combined with results of similar studies in other populations, including African-Americans (7), Ashkenazi Jews (16), British (18), Chinese (23), European Americans (7, 8), Finnish (9, 13, 15, 17), French (24), Han Chinese (23), Mexican Americans (6, 7, 10), and native Americans from the United States and Canada (11, 12). However, most of these scans have failed to generate highly significant linkage results. Replications of certain loci of these different scans can therefore direct further investigations toward positional cloning, targeting the most promising loci.

Our genome scan of a stratified sample of 146 Dutch families with type 2 diabetes mellitus, using nonparametric linkage analysis and exclusion mapping, revealed modest indications of linkage to regions on chromosome regions 2q12 (multipoint LOD score of 1.5) and 19q13 (multipoint LOD score of 1.3). Although we identified interesting LOD scores on chromosome 2, our locus mapped outside the previously described CAPN10 region on chromosome 2 (10, 19, 25). This locus lies approximately 105 cM distal of the locus found in our population. The locus found on chromosome 19 has not previously been described in the literature.

The results on chromosome 18 in the DS-20% group indicate linkage between D18S471 and D18S843 (multipoint LOD score of 2.3). The significance of this LOD score was evaluated using permutation analysis, with only 282 of 10,000 replicates yielded a LOD score higher than 2.3, giving a genome-wide significance of $P = 0.028$. This finding on chromosome 18 clearly replicates the linkage to this region previously reported in a Finnish-Swedish population (15) and in a confined isolated population of Dutch Caucasians near the town of Breda (Prof. B. A. Oostra, Erasmus University Rotterdam, personal communication). This is interesting because it suggests that our finding is genuine, because our stratified group (DS-20%) consists of only 44 affected sibling pairs.

Replication of linkage results from additional populations, whether as extension or follow-up studies in the same population or as independent genome scans in different populations, may provide vital confirmation of the original findings. Guidelines have been proposed for the level of significance necessary in sibling pair studies to show replication (26, 27). As for the significance of our finding, with respect to the null hypothesis (no disease susceptibility locus present in the study sample), Lander and Kruglyak (26) defined suggestive linkage for a LOD score of 2.2 (single point P value = 0.001) or higher, whereas significant linkage is defined only when the LOD score is 3.6 (single point P value = 2×10^{-5}) or higher. The LOD score obtained on

chromosome 18p11 (in DS-20%) meets the criteria for suggestive linkage. Although the loci found on chromosome region 2q12 and chromosome region 19q13 do not achieve suggestive LOD scores, they may indicate the existence of novel loci with minor effects involved in type 2 diabetes mellitus in the Dutch population. Alternatively, these findings on chromosome 2q and 19q await replication by others, to determine whether these signals are true linkages or false positives.

Apart from the findings on chromosomes 2, 18, and 19, our genome scan did not provide any supporting evidence for the other linkage findings described earlier. Several reports have described linkage to chromosomes 1q (8, 18, 24, 28, 29), 12q (30, 31), and 20q (8, 9, 16, 30). We observed no evidence for linkage to any of these three chromosome regions in our study.

This absence of strong agreement among genome-wide scans in type 2 diabetes mellitus is not unexpected (in part, because of the complexity of the disease involved). Gene discovery in complex diseases has been limited by substantial etiological and genetic heterogeneity, the possibility of genes of small effect, the interaction of multiple genes with each other and environmental factors, and the need for large sample sizes (32). A typical 10-cM genome scan fails to capture a significant proportion of the inheritance information, especially in cases of small sibships and lack of parental genotype information, as is usually the case in late-onset disorders such as type 2 diabetes mellitus (18). The inclusion of additional siblings to reconstruct parental haplotypes may compensate for part of the lost information. A two-stage screening design, with denser mapping in regions of interest identified by the primary low-resolution scan, has also been proposed, to enhance power by recovering some of the missed information. However, this approach may still miss regions of linkage if the evidence for linkage has, by chance, been underestimated in the primary scan, such that thresholds for dense mapping were not achieved (18). On the other hand, as shown in this study and others (15), sub-phenotyping of the disease may lead to substantial improvement in the results from genome-wide scans for type 2 diabetes mellitus.

The role of a susceptibility locus for type 2 diabetes mellitus on chromosome 18p11 and BMI, suggested by Parker *et al.* (15) and independently replicated in our Dutch population, is not known. It has often been suggested that obesity plays a causal role in the development of type 2 diabetes mellitus and is the most important determinant of type 2 diabetes mellitus (33). So far, the region on chromosome 18p11 has not been found in genome-wide scans for obesity. The association between type 2 diabetes mellitus and obesity is presumably attributable to multiple mechanisms, including elevations in plasma free fatty acids and TNF α released from so-called full adipocytes (34–37). Additional support for this hypothesis was seen in the recent demonstration of significantly increased B-cell apoptosis in obese *vs.* lean Zucker Diabetes Fatty rats (38).

Whether this chromosome 18p11 locus is a primary obesity locus or a locus which is important for the development of type 2 diabetes mellitus in already overweight individuals is a question for the future. Substantial new research will be

required to resolve this issue. Analysis of the region on chromosome 18 in the sequence databases revealed no obvious candidate gene. The chromosomal 18p11 region contains 111 transcripts, of which 61 are of unknown function and 50 either resemble a known function or have already been described. Much genetic analysis will have to be carried out to implicate the correct candidate gene.

In conclusion, our results indicate that a novel gene resides in the 18p11 region, which forms part of an as yet unidentified pathway involved in type 2 diabetes mellitus and obesity, and that there are indications of two novel loci on chromosome 2 and 19, both with minor effects involved in the development of type 2 diabetes mellitus in the Dutch population.

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† This paper is dedicated to the memory of Lodewijk Sandkuijl (1953–2002). He was a world expert on biostatistics and an inspiration to us.

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