Brief Genetics Report

A Genome-Wide Search for Genes Involved in Type 2 Diabetes in a Recently Genetically Isolated Population From the Netherlands

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Multiple genes, interacting with the environment, contribute to the susceptibility to type 2 diabetes. We performed a genome-wide search to localize type 2 diabetes susceptibility genes in a recently genetically isolated population in the Netherlands. We identified 79 nuclear families with type 2 diabetes who were related within 13 generations and performed a 770-marker genome-wide scan search for shared founder alleles. Twenty-six markers yielded a logarithm of odds (LOD) score >0.59 (nominal $P < 0.05$), of which 7 reached LOD scores >1.17 (nominal $P < 0.01$). The strongest evidence for a type 2 diabetes locus was at marker D18S63 on chromosome 18p (LOD 2.3, $P = 0.0006$). This region was investigated further using additional markers. For one of these markers (D18S1105), we found a significant association with type 2 diabetes (odds ratio 6.7 [95% CI 1.5–30.7], $P = 0.005$ for the 97-bp allele, assuming a dominant model), which increased when limiting the analysis to patients with high BMI (12.25 [2.1–71], $P = 0.003$). A locus on chromosome 18p in patients with high BMI was suggested earlier by Parker et al. Our study is the first to confirm this locus. *Diabetes* 52: 3001–3004, 2003

Type 2 diabetes has a substantial genetic component. Although different genes involved in type 2 diabetes have been localized (1), the genetic origin of the disease is unknown for most patients. It is most likely that a large number of genes are involved in type 2 diabetes. The effect of these genes may largely depend on the interaction with other genes and nongenetic factors. Genes for complex diseases such as type 2 diabetes are expected to be more easily identified in isolated populations than in a general outbred population (2). As a consequence of drift and founder effects, a large number of patients in isolated populations have likely inherited the disease susceptibility from a common genetic ancestor. Since adjacent markers on a chromosome are often transmitted together, patients who have inherited a susceptibility gene from a common ancestor are likely to share considerable stretches of DNA around the disease gene, leading to association of multiple adjacent markers to the disease. These regions can be traced relatively easily in a genome screen, particularly in populations of recent isolation (3). Although there is an ongoing debate whether genetically isolated populations are more suitable than outbred populations for genetic association studies, several studies in isolates have been successful. This concerns studies of complex disorders, such as multiple sclerosis, hypercholesteremia, and osteoarthritis, in various isolated populations, including the Finnish and Icelandic populations and inbred populations such as the Pima Indians (4–7).

We carried out a study aiming to find new genes involved in type 2 diabetes in a population of recent isolation. We ascertained 128 type 2 diabetic patients in an isolated village in the southwest of the Netherlands. This village was founded in the middle of the 18th century by ~150 people and was isolated until the last few decades (immigration <5%). From 1848 onward, the population expanded from 700 to 20,000 inhabitants. We identified 128 patients with type 2 diabetes who derived from 86 nuclear families. Genealogical information was collected up to 15 generations and revealed that 79 families (117 patients, 91% of all patients) could be traced to a common ancestor within 13 generations. When drawing 100 samples of "control subjects" who were randomly selected from our genealogic database and age and sex matched with study probands, on average, 38.9 ± 0.43 (45 ± 0.5%) people could be connected to a common ancestor, with a maximum of 50 (58%). Thus, the pedigree of the type 2 diabetic patients suggests that the disease may be in part explained by a rare mutation segregating in the isolate. In Fig. 1, the genealogical lineages of these patients are shown based on

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† Deceased. This study is dedicated to the memory of Lodewijk A. Sandkuijl.

LOD, logarithm of odds.

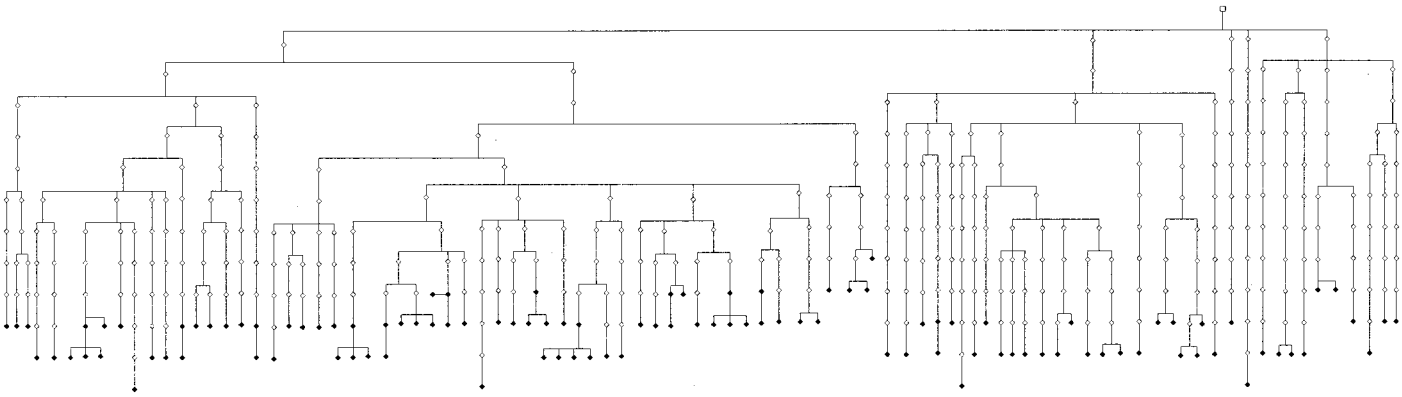

FIG. 1. Genealogical lineages of 117 type 2 diabetic patients who could be traced to a common ancestor within 13 generations. The figure is based on the shortest number of meiosis separating each person from this common ancestor.

the shortest number of meioses separating them. To ensure a more homogeneous study population, the analyses in this study were restricted to the 79 related families. The characteristics of the 117 patients are given in Table 1.

We performed a genome scan on these 117 type 2 diabetic patients and 202 first-degree relatives using 700 short tandem repeat markers. To evaluate statistical evidence for a type 2 diabetes locus, we used a combined linkage- and association-based approach (see RESEARCH DESIGN AND METHODS). The 202 first-degree relatives (spouses, parents, siblings, or children) were included to obtain control alleles for association analysis and gather linkage information. Furthermore, empirical *P* values were estimated using simulation analysis. A total of 26 markers showed nominal (maximum logarithm of odds [LOD] score >0.59, $P < 0.05$) evidence for linkage in presence of association with type 2 diabetes (Table 2). Seven markers (D1S2846, D2S323, D3S1270, D3S3681, D6S1569, D14S283, and D18S63) reached LOD scores >1.17 (nominal $P < 0.01$). The strongest evidence for a type 2 diabetes locus was obtained at 18p for marker D18S63 (LOD score 2.3, nominal $P = 0.0006$). For this marker, the empirical *P* value was estimated to be <0.005. This was the only location with an LOD >2. Also, one pair of markers located on chromosome 3 (D3S3567 and D3S3521, separated by less than 10 cM) yielded an LOD score >0.59. Between D3S3567 and D3S3521, one additional marker (D3S1277) showed weak evidence for association (LOD 0.43, $P = 0.08$). Although we cannot exclude a type 2 diabetes gene in this region, the evidence is rather weak compared with that for locus on 18p.

TABLE 1
Characteristics of 117 type 2 diabetic patients

n	117
Male/female	47/70
Age at examination (years)	63.5 ± 13.0
Age at onset (years)	57.9 ± 12.3
BMI (kg/m^2)	29.8 ± 4.8
Medication	
None	25
Oral	72
Insulin	20
Fasting glucose (mmol/l)	8.7 ± 3.2*

Data are means ± SD. *Includes subjects using glucose-lowering medication.

We explored the most promising region (18p) in more detail by investigating seven additional markers (Table 3). Although these analyses cannot be viewed as an independent confirmation, they are of interest to refine the region of interest encompassing the gene. Association of various additional markers with type 2 diabetes was observed. In particular, the odds ratio (OR) for the 97-bp allele of marker D18S1105 was high (6.7 [95% CI 1.5–30.7]) (Table 3). The significance of this finding was high ($P = 0.005$, not adjusted for multiple testing, dominant model). An evaluation of the ORs for D18S1105 and D18S63 showed that the effect of the risk-associated allele appears to be dominant in that the risk of type 2 diabetes was increased for both homo- and heterozygous individuals. However, the power of our study was limited, which is indicated by the wide range of the CIs shown in Table 3. Following Parker et al. (8), who found evidence for a type 2 diabetes locus in the same 18p region, we have conducted a separate analysis in those subjects who were in the upper 25% level of BMI. Although this substantially reduced the number of patients, the *P* value for the DS1105 remained low and the OR increased for 8 of the 10 markers shown in Table 3.

Although several genetic factors have been identified for type 2 diabetes, including mutations in the *HNF-1α*, *HNF-1β*, *HNF-4α*, and *IPF-1* genes involved in maturity-onset diabetes of the young (9), the genetic origin for the majority of patients with diabetes with adult onset is still unknown. Linkage studies for loci influencing the risk for type 2 diabetes have been conducted in a number of populations (reviewed in 1). In 1996, Hanis et al. (10) reported significant evidence for linkage of type 2 diabetes to the distal arm of chromosome 2. Using a linkage-disequilibrium approach similar to the approach used in our study, Horikawa et al. (11) demonstrated that susceptibility at this locus is confined to combinations of polymorphisms in the gene encoding calpain-10. Our study did not show evidence for association of markers in the calpain-10 region to type 2 diabetes. The strongest evidence for a type 2 diabetes locus was obtained at 18p, thus confirming the findings of Parker et al. (8).

RESEARCH DESIGN AND METHODS

Patients with type 2 diabetes were recruited at local health care centers and the Diabetes Service Breda, which is a regional clinical and laboratory service for the region. Since 1990, the Diabetes Service Breda has collected clinical and biochemical data on >8,000 patients with type 2 diabetes. All patients

TABLE 2
Markers with LOD score ≥0.59 ($P \leq 0.05$) in the initial genome scan

Chromosome	Map location (cM)*	Marker	λ	LOD score	P_A†	$P_E \pm$ SE
1	91.89	D1S2846	0.70	1.50	0.004	0.015 ± 0.009
2	5.4	D2S323	0.66	1.60	0.003	0.010 ± 0.007
3	6.96	D3S1270	0.59	1.30	0.007	0.011 ± 0.008
3	56.69	D3S3567	0.36	0.70	0.036	0.011 ± 0.008
3	63.12	D3S3521	0.23	0.97	0.017	<0.005
3	109.22	D3S3681	0.22	1.20	0.009	0.005 ± 0.005
4	33.42	D4S419	0.28	0.82	0.026	0.010 ± 0.007
4	83.02	D4S428	0.27	0.60	0.048	0.032 ± 0.022
4	96.58	D4S398	0.28	0.77	0.030	<0.005
4	206.58	D4S413	0.34	0.66	0.041	0.010 ± 0.007
6	141.15	D6S1569	0.39	1.30	0.007	<0.005
8	30.5	D8S552	0.46	0.78	0.029	0.005 ± 0.005
9	132.09	D9S1682	0.30	1.00	0.016	0.005 ± 0.005
10	170.94	D10S212	0.56	1.10	0.012	0.010 ± 0.007
11	153.31	D11S925	0.24	0.80	0.027	0.041 ± 0.014
11	138.56	D11S4126	0.59	0.89	0.021	<0.005
14	13.89	D14S283	0.33	1.60	0.003	<0.005
15	35.95	D15S1012	0.27	0.80	0.027	0.010 ± 0.007
17	36.14	D17S921	0.32	0.66	0.041	0.021 ± 0.010
17	58.25	D17S927	0.36	0.62	0.046	0.010 ± 0.007
18	8.3	D18S63	0.45	2.30	0.001	<0.005
18	96.48	D18S68	0.31	1.10	0.012	0.010 ± 0.007
18	114.26	D18S1161	0.35	0.94	0.019	<0.005
19	32.39	D19S865	0.26	0.86	0.023	<0.005
20	35.51	D20S898	0.32	0.59	0.050	0.015 ± 0.009
21	35.45	D21S1252	0.21	0.83	0.025	0.005 ± 0.005

*Based on Marshfield genetic map. †P_A, asymptotic P values; P_E, empirical P values.

underwent clinical and laboratory evaluations for their diabetes at regular 3-month intervals. We recruited 160 patients diagnosed with type 2 diabetes living in the isolated village. The overall participation rate was 80% ($n = 128$). The mean age at diagnosis of these patients was 57.9 years. All participants completed a questionnaire on family and medical history, underwent anthropometric and blood pressure measurements, and gave blood samples for DNA extraction and fasting serum. We applied the American Diabetes Association criteria for the diagnosis of diabetes to confirm the diagnosis (12). Individuals who were pregnant at the time of diagnosis or individuals who were insulin dependent within 1 year after diagnosis were assigned a diagnosis of "unknown." The study was approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam, and written consent was obtained from

TABLE 3
Association of markers in the chromosome 18p region with type 2 diabetes using a set of 71 case and 34 control subjects.

Marker	Position (kb)*	N†	Associated allele (mobility)	Frequency		OR (95% CI) [P value FET‡]		
				Case subjects	Control subjects	Heterozygous	Homozygous	Dominant
d18s1140	1,018	5	268	0.75	0.68	1.10 (0.26–4.57) [1.000]	1.67 (0.41–6.70) [0.476]	1.40 (0.37–5.33) [0.726]
d18s59	1,063	7	166	0.25	0.20	1.75 (0.70–4.38) [0.267]	1.13 (0.19–6.66) [1.000]	1.64 (0.69–3.88) [0.291]
d18s1105	2,251	5	97	0.17	0.03	6.39 (1.39–29.34) [0.009]	−[1.000]§	6.70 (1.46–30.67) [0.005]
d18s476	2,595	5	277	0.56	0.46	0.91 (0.30–2.70) [1.000]	2.20 (0.58–8.30) [0.319]	1.21 (0.43–3.44) [0.789]
d18s1098	3,287	4	171	0.42	0.40	1.34 (0.54–3.35) [0.640]	1.08 (0.32–3.63) [1.000]	1.27 (0.54–2.99) [0.590]
d18s481	3,484	7	185	0.11	0.08	1.35 (0.44–4.16) [0.783]	−[1.000]	1.45 (0.47–4.44) [0.596]
d18s63	3,860	6	86	0.44	0.32	2.59 (1.04–6.46) [0.063]	2.32 (0.62–8.70) [0.345]	2.53 (1.07–5.98) [0.045]
d18s459	3,956	7	140	0.20	0.14	1.87 (0.70–4.97) [0.248]	1.14 (0.10–13.17) [1.000]	1.78 (0.70–4.52) [0.264]
d18s1154	4,317	6	271	0.32	0.27	1.03 (0.43–2.46) [1.000]	2.06 (0.39–10.80) [0.480]	1.16 (0.51–2.66) [0.833]
d18s1132	5,046	5	122	0.46	0.36	1.20 (0.46–3.10) [0.810]	1.98 (0.64–6.08) [0.282]	1.46 (0.62–3.41) [0.393]
Using only 25% most obese subjects								
d18s1140	1,018	5	268	0.83	0.68	0.86 (0.07–10.67) [1.000]	2.75 (0.27–28.04) [0.626]	1.87 (0.19–18.27) [1.000]
d18s59	1,063	7	166	0.28	0.20	3.14 (0.90–11.03) [0.113]	0.00 [1.000]	2.57 (0.76–8.75) [0.215]
d18s1105	2,251	5	97	0.27	0.03	10.5 (1.77–62.44) [0.008]	−[0.243]	12.25 (2.11–70.99) [0.003]
d18s476	2,595	5	277	0.64	0.46	1.31 (0.21–8.18) [1.000]	4.20 (0.59–30.10) [0.197]	2.00 (0.36–11.22) [0.692]
d18s1098	3,287	4	171	0.43	0.40	3.18 (0.73–13.92) [0.180]	0.72 (0.62–8.46) [1.000]	2.48 (0.59–9.47) [0.324]
d18s481	3,484	7	185	0.07	0.08	0.86 (0.15–5.04) [1.000]	−[1.000]	0.86 (0.15–5.04) [1.000]
d18s63	3,860	6	86	0.47	0.32	4.51 (1.04–19.66) [0.052]	2.67 (0.33–21.73) [0.562]	4.08 (0.98–17.03) [0.063]
d18s459	3,956	7	140	0.10	0.14	0.89 (0.20–4.07) [1.000]	0.00 [1.000]	0.78 (0.18–3.48) [1.000]
d18s1154	4,317	6	271	0.33	0.27	1.04 (0.28–3.82) [1.000]	2.43 (0.28–20.82) [0.574]	1.21 (0.36–4.12) [1.000]
d18s1132	5,046	5	122	0.50	0.36	1.50 (0.37–6.14) [0.724]	2.50 (0.53–11.89) [0.423]	1.83 (0.52–6.46) [0.375]

Markers used in the original scan are in bold. *Based on Celera physical map; †Alleles at marker locus; ‡P value from Fisher exact test (FET), not adjusted for multiple testing; §OR could not be calculated (missing data).

all subjects. From 47 spouses of probands, 34 agreed to donate blood for DNA studies.

Collection of genealogical information. To determine which of the subjects descended from the original founders of the isolated village, a genealogical search was completed for each patient using church and municipal records of births, marriages, and deaths. Genealogical lineages for each patient were traced back 15 generations. Patients were linked to each other under the restriction of connecting as many individuals as possible to a common ancestor.

Genotyping. The genome screen was performed using 770 markers covering the whole genome. No gap was >18.7 cM and no more than 21 gaps (2.7%) were >10 cM.

Statistical analysis. A stepwise procedure was used in the analyses. Earlier, we used this approach in a study of type 1 diabetes (13). First, a test for linkage disequilibrium of single markers with diabetes was performed. The test used is based on a modification of the method described by Terwilliger (14) and assumes that one marker allele will be overrepresented on chromosomes that carry the disease mutation when many of these chromosomes descend from a single ancestor. The proportion of disease chromosomes with this ancestral allele is represented by the λ parameter. It is not known a priori which marker allele will be the overrepresented allele, and therefore the test considers each of the marker alleles separately as potential founder alleles. Consequently, a total likelihood is obtained for a given value of λ by computing the likelihood on the data for each potential founder allele and summing those likelihoods, which are weighted for the population frequency of the respective founder allele. As this procedure implies estimation of a single parameter, λ, which is restricted to be positive or zero, under the null hypothesis the test statistics are distributed as one-tailed χ^2 test with 1 degree of freedom (14). While the procedure was originally applied to genotype data in samples of affected and unaffected individuals, it can also be used to calculate likelihood for pedigree data. The approach we adopted is based on the standard model-based methodology, as realized in the LINKAGE package of programs. However, the model was extended to allow for association by incorporating the λ parameter into the model (15). In the estimation procedure the recombination fraction was fixed at 0.01 and the disease gene frequency was kept constant at 2.5%, a dominant model was assumed (Fig. 1). Subjects were assigned to one of the age-based liability classes, which are derived from the age-of-onset distribution of patients with type 2 diabetes from the Rotterdam study, a population-based cohort study in the Netherlands. In this study, an LOD score >0.59 (corresponding to nominal $P < 0.05$) was used as a threshold to select markers for further discussion. To obtain empirical *P* values, a simulation study was conducted. For every marker listed in Table 2, we used MERLIN (16) to generate 200 samples. Every sample was then reanalyzed using our approach.

In the second phase, additional markers were accessed for the region surrounding D18S63, which showed the strongest evidence for association in the initial genome scan. A case-control analysis was performed to test for association of additional markers with type 2 diabetes. For the case-control analysis, one proband was selected per family. We used healthy spouses of probands as control subjects. The relative risk for each ancestral allele was estimated as an OR with a 95% CI.

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