Mapping Genes for NIDDM

Design of the Finland–United States Investigation of NIDDM Genetics (FUSION) Study

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OBJECTIVE — To map and identify susceptibility genes for NIDDM and for the intermediate quantitative traits associated with NIDDM.

RESEARCH DESIGN AND METHODS — We describe the methodology and sample of the Finland–United States Investigation of NIDDM Genetics (FUSION) study. The whole genome search approach is being applied in studies of several different ethnic groups to locate susceptibility genes for NIDDM. Detailed description of the study materials and designs of such studies are important, particularly when comparing the findings in these studies and when combining different data sets.

RESULTS — Using a careful selection strategy, we have ascertained 495 families with confirmed NIDDM in at least two siblings and no history of IDDM among the first-degree relatives. These families were chosen from more than 22,000 NIDDM patients, representative of patients with NIDDM in the Finnish population. In a subset of families, a spouse and offspring were sampled, and they participated in a frequently sampled intravenous glucose tolerance test (FSIGT) analyzed with the Minimal Model. An FSIGT was completed successfully for at least two nondiabetic offspring in 156 families with a confirmed nondiabetic spouse and no history of IDDM in first-degree relatives.

CONCLUSIONS — Our work demonstrates the feasibility of collecting a large number of affected sib-pair families with NIDDM to provide data that will enable a whole genome search approach, including linkage analysis.

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Abbreviations: ASP, affected sib pair; CV, coefficient of variation; FSIGT, frequently sampled intravenous glucose tolerance test; FUSION, Finland–United States Investigation of NIDDM Genetics; IBD, identical by descent; LOD, logarithm of odds; NHDR, National Hospital Discharge Registry; OGTT, oral glucose tolerance test; OHA, oral hypoglycemic agent; QC, quality control; RIA, radioimmunoassay; WHO, World Health Organization.

NIDDM is a multifactorial disease that is increasingly common in adults in most populations (1). The familial character of NIDDM has long been suspected and was properly documented by several studies early in the 20th century (2–5). In the past 2 decades, twin studies and family studies have provided strong evidence for a genetic component in NIDDM. Concordance rate estimates for monozygotic twins range from 34 to 100% and are approximately two times higher than those for dizygotic twins (6–9). Family studies suggest a three- to fourfold increased risk to first-degree relatives of individuals with NIDDM compared with the general population (10).

Because of the likely etiologic heterogeneity of NIDDM, it may be useful to study intermediate quantitative traits that predispose individuals to diabetes in order to identify the diabetes genes (11). Familial aggregation of insulin resistance has been shown in several studies (12–16), and aggregation has recently been demonstrated for acute insulin secretory response (17). Although it remains uncertain whether the primary defect leading to NIDDM is peripheral insulin resistance or a relative decrease in insulin secretion, most NIDDM patients have both defects. Thus, efforts to map these and other NIDDM-related quantitative traits have the potential to increase our understanding of the genetics of NIDDM.

Until recently, the search for genes for NIDDM has focused on population association and linkage studies using the candidate-gene approach. Although association or linkage has been reported for some candidate genes, the results from these studies have not elucidated diabetes genes and instead indicate that a systematic search of the entire genome is warranted. Performing a genome search now is propitious because increasingly dense and more complete genetic maps have become available, methods of genotyping have become more efficient, and linkage analysis techniques suited to the study of complex diseases continue to be developed (11).

To map the genes for NIDDM and NIDDM-associated traits such as acute

insulin response and insulin resistance, we have chosen the Finnish population as the target for our study. In this paper, we describe the rationale, design, and sample characteristics of the Finland–United States Investigation of NIDDM Genetics (FUSION) study.

RESEARCH DESIGN AND METHODS

Family ascertainment criteria

Relatives of individuals who were diagnosed with NIDDM at an early age have a higher disease risk than relatives of individuals with a later onset of disease (18). However, there is a relatively greater risk of IDDM among the younger age-groups. Therefore, to maximize the genetic component while minimizing the likelihood of identifying subjects with IDDM, we ascertained probands in whom diabetes was diagnosed between 35 and 60 years of age. A given family was eligible for participation in the FUSION study if *1*) the proband or another affected sibling was diagnosed with NIDDM between 35 and 60 years of age, *2*) there was no history of IDDM in first-degree relatives, *3*) the proband had one or more full siblings diagnosed with NIDDM at any age, and *4*) at least one parent was apparently nondiabetic, with preference given to families with living parents or parents who had lived a long life without known diabetes. The last criterion was included to minimize bilineality. In this paper, we use the term index case to indicate the first proband or affected sibling in each family who met our study inclusion criteria.

Sources and types of families

There are approximately 150,000 known NIDDM patients in Finland. The probands in the FUSION study were identified primarily from the National Hospital Discharge Registry (NHDR), which includes records since 1970 of all hospitalized patients with diabetes, and from previous studies carried out by the National Public Health Institute in Finland. From the NHDR, we identified all patients who were hospitalized with a diagnosis of NIDDM in Finland during 1987–1993.

A total of 33,029 potential probands were initially identified in 1994, 29,807 of them from the NHDR. A total of 22,312 screening questionnaires were sent to the NIDDM patients living relatively close to one of the 21 clinics we established for this study. The patients were also sent a short description of study eligibility criteria, including our minimum requirement of a diabetic sib pair. A total of 9,626 individuals returned the screening questionnaire. In addition, 14 eligible families voluntarily contacted us and were included in the study.

Families in the FUSION study were classified as nuclear or extended. In the nuclear families, all the affected siblings and any living parents were studied. At least one affected sib pair (ASP) was required in each family. In the extended families, in addition to the nuclear family members, we studied a nondiabetic spouse and at least two offspring of an affected sibling, fulfilling the proband criteria described above.

Control subjects

The primary purpose of the control subjects in the FUSION study was to provide allele frequency estimates in unaffected individuals for comparison with affected individuals. All our control subjects were born in the year 1925 and had been examined with oral glucose tolerance tests (OGTTs) in 1990 as part of the South Finland Aging Study (19). Those who had normoglycemic OGTT values by the World Health Organization's (WHO) criteria were given a second OGTT as part of the FUSION study in 1995, and the 248 individuals with normal glucose tolerance according to both OGTTs were included as control subjects for the FUSION study.

Diabetes status

The diagnosis of diabetes was accepted if an individual was receiving drug treatment for diabetes or if the blood glucose values obtained from the medical records met current WHO criteria (20). Fasting blood glucose was determined in all individuals at the FUSION examination. If patients were treated with diet alone and fasting blood glucose was <7.0 mmol/l, the diagnosis of diabetes was confirmed by OGTT. The age at diagnosis of NIDDM was based on self-reported information. The inferred diabetes status of parents was based primarily on information reported by the affected siblings because nearly all their parents were deceased.

Cases of probable IDDM were identified to exclude them from future analyses. An individual was classified as having probable adult-onset IDDM if *1*) insulin treatment was started within 10 years of disease diagnosis, autoantibodies to GAD were detected, and the fasting C-peptide was ≤ 0.30 nmol/l; or if *2*) insulin treatment was started within 4 years of diagnosis and the fasting C-peptide was ≤ 0.30 nmol/l. Medical record review was carried out for all affected individuals whose C-peptide was <0.50 nmol/l, who had antibodies to GAD, or who were treated with insulin and for whom C-peptide and/or GAD antibody samples were not assayed in the FUSION study to confirm their age of diagnosis and the age when insulin treatment was started.

Study clinics and examinations

The FUSION study was carried out in 21 cities distributed throughout much of Finland. The study nurses from each clinic attended a 1-week training seminar in Helsinki. Blood collection and all clinical measurements were carefully standardized.

All study subjects underwent a screening examination during a single visit. In addition, nondiabetic spouses and offspring returned for a frequently sampled intravenous glucose tolerance test (FSIGT) during a second visit. At the screening examination, blood pressure, pulse, weight, height, and waist and hip circumferences were measured on all participating family members and control subjects. Also, data on medical and lifestyle history, medication, current health status, physical activity, diet, smoking history, alcohol consumption, gestational medical history, and birth weight were collected from all family members and control subjects.

Blood samples for DNA, fasting glucose and insulin, and serum lipids were drawn for all participating family members. In addition, OGTTs were carried out in nondiabetic family members. OGTTs were performed according to WHO criteria (75-g oral load of glucose). Serum C-peptide and autoantibodies to GAD were assayed in the affected family members. An overnight urine sample was collected from each diabetic family member to assess urinary albumin-to-creatinine ratio.

Tolbutamide-modified FSIGTs were performed in nondiabetic spouses and nondiabetic offspring during a second visit in six clinics. The reduced sampling protocol, in which 14 blood samples for glucose and insulin were collected over the course of a 3-h test (21), was employed. FSIGT data were analyzed, using the Minimal Model method (22), to derive quantitative measures of insulin sensitivity (S_I) and glucose effectiveness (S_G). Insulin secretion was assessed as the acute insulin response

to glucose (AIR_G) calculated as described by Ward et al. (23).

Assays

Blood glucose concentration was initially measured at the study clinic using a glucose monitoring device (One Touch; LifeScan, Milpitas, CA). Plasma glucose, plasma insulin, and serum C-peptide samples were frozen and shipped on dry ice to the central laboratory at the University of Southern California in Los Angeles, California, for measurement. Plasma glucose was measured on an autoanalyzer (YSI-2700; YSI, Yellow Springs, OH) using the glucose oxidase method. Interassay coefficients of variation (CVs) were 2.9 and 2.3% for the low and high quality control (QC) pools, respectively. The correlation between One Touch and plasma glucose values was 0.94 and 0.96 for fasting glucose and postload glucose values, respectively. Plasma insulin was measured by radioimmunoassay (RIA) using dextran-charcoal separation (24). Antibody and tracer for the insulin RIA were purchased from Novo Nordisk (Bagsvaerd, Denmark). Interassay CVs were 11 and 13% for low and high QC pools, respectively. Serum C-peptide was determined by using a kit purchased from Linco (St. Charles, MO) with interassay CVs of 14 and 5% for low and high QC pools, respectively.

Autoantibodies to GAD were measured at the University of Washington in Seattle, Washington, by fluid-phase immunoprecipitation assay (25) using recombinant human GAD65 radiolabeled by in vitro translation (TNT; Promega, Madison, WI) in the presence of [^{35}S]methionine (Amersham, Arlington Heights, IL) (26). The assay had high sensitivity and 100% specificity in an international GAD antibody workshop (27) and an interassay CV of 15%.

Serum total and HDL cholesterol and triglycerides were determined in Helsinki from fresh samples using an enzymatic assay method (CHOD-PAP; Monotest; Boehringer Mannheim, Indianapolis, IN). The interassay CVs for total cholesterol, HDL cholesterol, and triglycerides were 1.2, 2.2, and 1.6%, respectively. Urine creatinine and albumin concentrations were also measured at the National Public Health Institute in Helsinki. The creatinine in urine was determined using the method of Jaffe (28), and the urinary albumin concentration was determined by the immunoturbidometric method, using reagents from Roche (Montclair, NJ) (29). Interassay CVs for urine creatinine and albumin were 2.4 and 5.1%, respectively.

DNA was isolated from 30 ml of fresh whole blood in EDTA using the Purgene D-50K DNA isolation kit (Gentra Systems, Plymouth, MN) at the National Public Health Institute. The isolated samples were frozen and shipped on dry ice to the National Human Genome Research Institute in Bethesda, Maryland.

Genotyping

The genetic markers being used for genetic mapping in the FUSION study are modified ABI sets (Applied Biosystems Division of Perkin-Elmer, Foster City, CA) consisting of approximately 365 dinucleotide repeats divided into 28 panels, each of which contains 7 to 19 markers generating a 10-centimorgan (cM) average density map. Incorrect genotypes can give rise to inflated map lengths and reduced power to detect linkage. Thus, a genotyping system that has a low frequency of errors is a particularly important tool in the study of complex diseases, in which gene effects may often be weak. Because the commercially based software available from ABI is not very efficient at automatically adjusting for gel-to-gel variation in allele sizing, or for allele calling ("binning"), we developed software to do these tasks automatically. In addition, we optimized our genotyping system for precise sizing and binning of alleles (30). The automation also allows us to run a series of internal checks to improve the binning accuracy and reproducibility. The blinded presence of samples in duplicate allowed us to estimate a genotype-specific error rate of 0.034% (2 of 5,846) for the last 64 markers typed in our laboratory.

Because of previous findings suggesting an association between HLA haplotypes and NIDDM, *HLA-A, C, B, Bw, DR* and *DQ* genotypes were also determined serologically using 180 HLA antisera (120 class I and 60 class II) (31) in 110 extended families in Helsinki.

Statistical analysis plan

The variety of phenotypic data, together with the uncertain etiology of NIDDM and its associated quantitative traits, requires a multifaceted statistical analysis strategy. In the initial phase of genome screening for NIDDM, we will depend primarily on mode-of-inheritance–free methods of linkage analysis that compare estimated allele sharing identical by descent (IBD) in the ASPs to expectations under the hypothesis that no disease locus is present (32–34). By parameterizing our models in terms of probability of IBD sharing in the ASPs, we will be able both to search for linkage and to build exclusion maps of regions that appear unlikely to harbor NIDDM genes of specified levels of genetic effect. Similar approaches can be taken for the NIDDM-associated quantitative traits using a regression or variance components framework (35,36).

In the initial genome scan, we will follow up even rather modest suggestions of linkage by typing additional markers in the region. Our simulations (32) suggest that following up logarithm of odds (LOD) scores even as low as 1.0 is not expected to result in more than about 10 false-positive findings in a genome scan of 300–400 markers and provides good power to detect loci of even modest effect. For regions identified as likely to harbor a disease gene, we also will employ mode-of-inheritance–based methods of linkage analysis as well as combined segregation and linkage analysis in hopes of more accurately assessing the existence and the effect of the putative disease loci. In addition, we will emphasize linkage disequilibrium mapping as a complementary strategy of disease gene localization, particularly once initial evidence for linkage is obtained.

RESULTS

Family ascertainment

Among the 9,626 respondents to the screening questionnaire, there were 1,835 potential probands with onset of diabetes between 35 and 60 years of age who had at least one affected sibling and not both parents known to be diabetic. These potential probands were ranked according to their family information, with preference given to families with evidence of at least one nondiabetic parent and multiple early-onset affected siblings. A total of 1,382 potential probands living near one of the 21 FUSION study clinics were contacted by phone to confirm their eligibility and willingness to participate. Of these, 470 potential probands or their affected siblings were unable or unwilling to participate. An additional 286 diabetic patients were found be ineligible during the first interview. Thus, from the list of potential probands and volunteers who contacted us, we enrolled 640 affected individuals from 577 separate families in the FUSION study. In addition, three families were enrolled in the study after the index case refused to participate.

Table 1—*Diabetes affection status in all individuals*

	Individuals studied	Confirmed NIDDM	Probable adult-onset IDDM	Nonaffected
Index case	577	550	20	7
Sibling	733	638	22	73
Father	10	4	0	6
Mother	32	16	1	15
Spouse	221	16	0	205
Offspring	555	16	0	539
Control	248	0	0	248
Total	2,376	1,240	43	1,093

Data are *n*.

Characteristics of the study subjects

All index cases, siblings, and control subjects were born in Finland. Of the 577 studied index cases, diabetes status could not be confirmed in 7 individuals by fasting plasma glucose, OGTT, or review of the medical records. In addition, 20 index cases were classified as having probable adult-onset IDDM, leaving 550 index cases meeting FUSION study criteria for NIDDM (Table 1). A total of 733 siblings of the index cases with self-reported history of NIDDM were studied, and 638 of these siblings met FUSION study criteria for NIDDM. A total of 2,376 individuals were examined in the FUSION study (Table 1).

In the FUSION study, we have investigated a total of 580 Finnish families, ranging in size from 1 to 13 members. Of this total, 533 families have at least two siblings affected with diabetes, resulting in a total of 795 ASPs (Table 2). In 495 of these 533 families, no cases of probable adult-onset IDDM were identified among the individuals examined in the FUSION study or among the first-degree relatives of the index cases or affected siblings, resulting in a total of 740 ASPs.

Offspring were studied in 210 extended families, and FSIGTs were carried out in 79% of these families. Altogether, 602 FSIGTs were successfully done for nonaffected family members in families with no IDDM cases. An FSIGT was completed for at least two nondiabetic offspring in 156 families with a nondiabetic spouse confirmed by OGTT (Table 2).

Of the affected index cases and affected siblings, 55 and 45% were men, respectively. The mean age of onset of diabetes was 49 years (range, 25–69) in male index cases and 51 years (30–72) in female index cases. The age of onset for affected siblings was 53 (29–74) and 54 (22– 80) years in men and women, respectively (Table 3).

Of the index cases and siblings with NIDDM, 12% were treated with diet alone, 46% with oral hypoglycemic agents (OHAs), 21% with insulin alone, and 20% with a combination of insulin and OHAs. The distribution of treatment modes corresponds well with that seen in Finnish diabetes clinics in general (37).

There were no notable differences in the means of the weight-related variables between affected index cases and siblings within either sex (Table 3). Of the male index cases and siblings, 40% were clearly overweight (BMI >30 kg/m^2); 51% of the female index cases and 44% of the female siblings with NIDDM were overweight. The control subjects were leaner: only 18% of men and 24% of women were clearly overweight.

Of the index cases and their affected siblings, 7% had a fasting C-peptide value of ≤ 0.30 nmol/l. Of these diabetic subjects, 42% were classified as having probable adult-onset IDDM. Antibodies to GAD were detected in 3.2% of the affected index cases and in 4.1% of the affected siblings. After excluding the subjects classified as having probable IDDM, 10 (1.9%) of the 517 affected index cases and 13 (2.2%) of the 594 affected siblings showed GAD antibody positivity. In normoglycemic control subjects, GAD autoantibodies were detected in 2 (1.7%) of the 120 studied individuals. The mean fasting C-peptide values between 1.5 and 1.7 nmol/l for affected index cases and siblings (Table 4), respectively, are typical for individuals with NIDDM.

There were no notable differences between index cases and siblings in serum lipid and blood pressure values (Table 5). HDL cholesterol levels were considerably higher, and triglyceride levels were lower, in control subjects than in affected individuals. Based on the questionnaire data, 60% of the male index cases and 51% of male

Table 2—*Distribution of the family sizes in the FUSION study*

	Studied family members (*n*)											Total number of families	Families with ≥2 siblings
	0	1	2	3	4	5	6	7	8	9	10+		
All family members													
Number of families	—	15	261	81	26	75	58	30	14	11	9	580*	
Affected siblings													
Number of families	—	39	431	92	7	1	1	1	—	—	—	572	533
After excluding families with IDDM	—	37	400	86	6	1	1	1	—	—	—	532	495
Offspring in extended families													
Number of families	—	14	108	50	23	8	6	1	—	—	—	210	
Offspring with sampled FSIGT†													
Number of families in which spouse also sampled	4	18	78	31	15	7	3	0	—	—	—	156	
Spouse not sampled	—	5	14	4	2	0	1	1	—	—	—	27	

Data are *n*. Control subjects were not included in these data. *Three index cases refused to participate after the family was enrolled; additional affected siblings met study criteria for proband in these families. †In families with no IDDM cases.

Table 3—*Age- and weight-related data in the subjects in families with no IDDM cases*

	Subjects (*n*)	Age at clinical examination (years)	Age at diabetes diagnosis (years)	Duration of diabetes (years)	BMI (kg/m^2)	Waist-to-hip ratio
Men						
Affected index cases	290	62.2 ± 7.1 (62.7)	49.4 ± 8.1 (50.0)	12.9 ± 7.0 (11.8)	29.3 ± 4.4 (28.8)	0.98 ± 0.06 (0.97)
Affected siblings	276	63.6 ± 8.7 (64.4)	53.3 ± 9.4 (53.0)	10.4 ± 7.2 (9.1)	29.3 ± 4.1 (29.1)	0.98 ± 0.06 (0.98)
Affected fathers	3	77.7 ± 6.0 (77.9)	55.5 ± 3.5 (55.5)	19.3 ± 0.9 (19.3)	24.8 ± 1.8 (24.0)	0.97 ± 0.01 (0.98)
Nonaffected fathers	4	77.8 ± 11.0 (76.9)	—	—	27.1 ± 3.5 (27.6)	0.96 ± 0.06 (0.97)
Nonaffected spouses	63	65.8 ± 8.2 (66.7)	—	—	27.6 ± 3.5 (27.9)	0.94 ± 0.06 (0.94)
Nonaffected offspring	260	35.0 ± 7.2 (35.7)	—	—	26.5 ± 4.0 (26.0)	0.92 ± 0.07 (0.92)
Control subjects	119	70.0 ± 0.3 (70.0)	—	—	27.0 ± 4.1 (26.6)	0.94 ± 0.06 (0.94)
Women						
Affected index cases	240	65.3 ± 7.6 (65.7)	50.8 ± 7.6 (50.5)	14.5 ± 7.0 (14.3)	30.8 ± 5.1 (30.0)	0.90 ± 0.07 (0.90)
Affected siblings	337	66.2 ± 8.6 (66.9)	54.3 ± 9.8 (55.0)	11.7 ± 8.1 (10.6)	30.0 ± 5.2 (29.5)	0.89 ± 0.07 (0.89)
Affected mothers	16	79.6 ± 5.7 (80.9)	67.9 ± 11.6 (67.0)	12.8 ± 9.4 (14.6)	27.0 ± 4.1 (26.8)	0.87 ± 0.05 (0.88)
Nonaffected mothers	10	79.5 ± 7.9 (79.5)	—	—	27.9 ±.3.1 (26.7)	0.91 ± 0.04 (0.92)
Nonaffected spouses	134	59.4 ± 6.7 (59.8)	—	—	28.9 ± 4.8 (28.2)	0.85 ± 0.07 (0.85)
Nonaffected offspring	260	34.3 ± 7.4 (34.7)	—	—	25.4 ± 4.9 (24.5)	0.80 ± 0.06 (0.80)
Control subjects	129	70.2 ± 0.3 (70.2)	—	—	27.3 ± 4.1 (26.3)	0.82 ± 0.05 (0.81)

Data are means ± SD (median).

affected siblings had hypertension; 25% of the male control subjects reported hypertension. In women, the contrast between affected individuals and control subjects was even greater: 65 and 60 vs. 24% for female index cases, affected siblings, and control subjects, respectively.

The Minimal Model–derived FSIGT values for nondiabetic spouses and offspring are shown in Table 6. Nondiabetic women were generally more insulin sensitive than nondiabetic men. In men, this finding was partly compensated for by a more robust acute insulin response. However, the mean disposition index remained higher in females than in males. Also, the nonaffected spouses were more insulin resistant than their offspring.

CONCLUSIONS — In a study of the determinants of a complex genetic disease

Table 4—*Plasma glucose, plasma insulin, and serum C-peptide values in subjects in families with no IDDM cases*

	OGTT				
	Fasting plasma glucose (mmol/l)	2-h plasma glucose (mmol/l)	Fasting plasma insulin (pmol/l)	2-h plasma insulin (pmol/l)	Fasting serum C-peptide (nmol/l)
Men					
Affected index cases	10.7 ± 3.5 (10.1)	—	104 ± 70 (90)	—	1.5 ± 1.0 (1.3)
Affected siblings	10.4 ± 3.4 (9.6)	—	108 ± 65 (96)	—	1.7 ± 0.9 (1.5)
Affected fathers	10.7 ± 6.0 (7.6)	—	60 ± 16 (54)	—	—
Nonaffected fathers	5.0 ± 1.8 (5.6)	5.8 ± 1.7 (6.0)	98 ± 73 (84)	508 ± 572 (282)	—
Nonaffected spouses	5.5 ± 0.9 (5.4)	6.3 ± 1.8 (6.0)	85 ± 71 (66)	462 ± 408 (336)	—
Nonaffected offspring	5.2 ± 0.6 (5.1)	5.2 ± 1.5 (4.9)	72 ± 39 (66)	300 ± 246 (207)	—
Control subjects	5.1 ± 0.5 (5.1)	5.6 ± 1.2 (5.5)	66 ± 37 (60)	365 ± 218 (348)	—
Women					
Affected index cases	10.7 ± 3.3 (10.4)	—	126 ± 72 (114)	—	1.6 ± 0.9 (1.4)
Affected siblings	10.0 ± 3.4 (9.5)	—	116 ± 63 (102)	—	1.6 ± 0.9 (1.5)
Affected mothers	8.0 ± 2.5 (7.4)	—	104 ± 58 (90)	—	—
Nonaffected mothers	5.8 ± 0.8 (5.7)	7.6 ± 2.1 (7.8)	92 ± 30 (84)	408 ± 193 (432)	—
Nonaffected spouses	5.2 ± 0.6 (5.1)	6.1 ± 1.7 (6.0)	71 ± 33 (66)	405 ± 259 (357)	—
Nonaffected offspring	4.9 ± 0.5 (4.8)	5.5 ± 1.4 (5.4)	62 ± 30 (54)	335 ± 244 (243)	—
Control subjects	4.9 ± 0.4 (4.9)	5.7 ± 1.2 (5.6)	67 ± 32 (60)	410 ± 238 (378)	—

Data are means ± SD (median).

Table 5—*Serum lipid and blood pressure values in studied subjects in families with no IDDM cases*

	Serum total cholesterol (mmol/l)	Serum HDL cholesterol (mmol/l)	Serum triglycerides (mmol/l)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Men					
Affected index cases	5.7 ± 1.3 (5.5)	1.03 ± 0.29 (1.00)	2.6 ± 2.3 (2.0)	152 ± 21 (150)	87 ± 11 (88)
Affected siblings	5.6 ± 1.3 (5.6)	1.02 ± 0.27 (1.00)	2.5 ± 2.6 (1.9)	150 ± 22 (148)	85 ± 12 (84)
Affected fathers	4.6 ± 1.7 (5.6)	1.12 ± 0.36 (1.11)	1.1 ± 0.6 (1.2)	151 ± 8 (152)	84 ± 5 (82)
Nonaffected fathers	5.5 ± 0.7 (5.7)	1.06 ± 0.14 (1.11)	1.4 ± 0.2 (1.5)	145 ± 30 (146)	83 ± 17 (85)
Nonaffected spouses	5.5 ± 0.8 (5.5)	1.12 ± 0.30 (1.07)	1.6 ± 0.9 (1.4)	150 ± 20 (148)	85 ± 11 (84)
Nonaffected offspring	5.3 ± 1.0 (5.2)	1.19 ± 0.29 (1.16)	1.6 ± 1.0 (1.2)	130 ± 13 (130)	82 ± 10 (80)
Controls subjects	5.7 ± 0.9 (5.6)	1.28 ± 0.29 (1.26)	1.5 ± 0.7 (1.4)	149 ± 18 (150)	88 ± 9 (88)
Women					
Affected index cases	5.9 ± 1.3 (5.8)	1.15 ± 0.32 (1.08)	2.5 ± 1.7 (2.1)	156 ± 24 (154)	83 ± 11 (82)
Affected siblings	5.9 ± 1.1 (5.8)	1.16 ± 0.30 (1.12)	2.3 ± 1.6 (1.8)	157 ± 25 (154)	84 ± 11 (84)
Affected mothers	5.7 ± 1.1 (5.9)	1.17 ± 0.25 (1.14)	2.4 ± 1.8 (1.8)	151 ± 20 (160)	79 ± 16 (80)
Nonaffected mothers	6.0 ± 1.4 (6.3)	1.38 ± 0.44 (1.36)	1.4 ± 0.4 (1.4)	143 ± 18 (134)	80 ± 6 (80)
Nonaffected spouses	6.1 ± 1.1 (6.0)	1.43 ± 0.35 (1.36)	1.4 ± 0.6 (1.2)	146 ± 22 (142)	86 ± 11 (86)
Nonaffected offspring	4.9 ± 0.9 (4.9)	1.36 ± 0.30 (1.34)	1.1 ± 0.5 (0.9)	123 ± 14 (120)	77 ± 10 (78)
Control subjects	6.4 ± 1.0 (6.4)	1.54 ± 0.30 (1.55)	1.4 ± 0.6 (1.2)	147 ± 16 (148)	86 ± 10 (86)

Data are means ± SD (median).

such as NIDDM, careful attention should be paid to all elements of study design. These elements include selection of the study population, ascertainment and exclusion criteria for probands and families, and the choice of phenotypes and genetic markers for study. Currently, there are several ongoing efforts to map the susceptibility genes for NIDDM using the whole genome search approach (38–42) and many others using the candidate-gene approach. To permit valid comparison of results from different studies and to assess the meaning of consistency or inconsistency of these results, proper understanding of study design, study methods, and family samples is of critical importance. In this report, we have described the sampling design and study methods we are using, and the sample of families obtained, in the FUSION study.

Advantages and relevance of a genetic study of NIDDM in Finland

There are several advantages in using a Finnish population for carrying out genetic studies. The vast majority of Finland's 5.1 million inhabitants belong linguistically and ethnically to the Finno-Ugrian ethnic group with some admixture with the Swedish population in the coastal areas of the country. Finns are closely related to other populations of northern Europe (43), and it has been estimated that the bottleneck in the Finnish population dates back 4,000 years (44). During the first centuries after the end of migration in about A.D. 800, linguistic, geographic, and cultural barriers kept the Finns isolated from the surrounding areas (45). This small founding population and relative homogeneity increases the likelihood that individual genes predisposing to NIDDM can be identified, as has been demonstrated for genes responsible for several monogenic hereditary diseases (46–48). Relative genetic homogeneity is likely to have resulted in fewer NIDDM genes segregating than in outbred populations such as those of the U.S. or the U.K., so that individual NIDDM-susceptibility genes should be easier to detect. This distinction is important, because the detection of genes for a complex disease generally requires large samples of families, and the power to detect linkage is inversely related to the magnitude of the effect of the gene on disease risk.

NIDDM in Finland is clinically similar to NIDDM in other European populations, and the prevalence of NIDDM in Finland is similar to that of most other European populations (49). Thus, it is reasonable to expect that NIDDM susceptibility genes identified

Table 6—*FSIGT variables in subjects in families with no IDDM cases*

	Subjects (*n*)	AIR_G (μU/ml × 10 min)	S_I (10^{-5} $min^{-1} \cdot pmol^{-1} \cdot l^{-1}$)	S_G (× 100 min^{-1})	Disposition index ($S_I \times AIR_G$)
Men					
Nonaffected spouses	49	239 ± 193 (169)	4.6 ± 2.6 (4.3)	1.6 ± 0.5 (1.5)	987 ± 789 (817)
Nonaffected offspring	233	225 ± 174 (189)	7.3 ± 4.4 (6.5)	1.6 ± 0.5 (1.6)	1340 ± 890 (1,158)
Women					
Nonaffected spouses	108	235 ± 156 (198)	6.4 ± 3.5 (5.8)	1.7 ± 0.6 (1.6)	1348 ± 913 (1,131)
Nonaffected offspring	212	206 ± 125 (177)	7.9 ± 4.6 (7.0)	1.9 ± 0.6 (1.8)	1465 ± 942 (1,327)

Data are means ± SD (median). AIR_G, acute insulin response to glucose; S_G, glucose effectiveness; S_I, insulin sensitivity.

in Finns will play a role in NIDDM risk in non-Finnish populations as well.

Previous studies

Until recently, genetic studies of NIDDM have focused on the candidate-gene approach. Such studies in Finland have included investigations of the HLA system on chromosome 6 (50), and genes for glycogen synthase (51), glucokinase (52,53), hexokinase II (54,55), GLUT4 (56), fatty acid binding protein (57), apolipoprotein D (58), the insulin receptor substrate 1 (59,60), and the β_3-adrenergic receptor (61). Except for a very significant association between certain HLA haplotypes and NIDDM (50), these studies have not revealed major positive findings.

Recently, results from two whole genome searches for NIDDM have been reported, one based on 330 Mexican-American ASP families from Starr County, Texas (38), the second on 26 moderate-sized Finnish families from western Finland (39). Hanis et al. (38) reported evidence for linkage in several chromosomal regions, with the strongest evidence at the anonymous marker D2S125 on chromosome 2q, and they were able to exclude the existence of a single major locus for NIDDM throughout most of the genome. Mahtani et al. (39) failed to find compelling evidence for linkage anywhere in the genome with their complete sample. However, when they restricted attention to the six families with the lowest mean 30-min insulin level during an OGTT among the affected individuals, they did find evidence for linkage to the MODY3 region of chromosome 12q.

Rationale for a whole genome search

We have chosen to carry out a whole genome search for genes for NIDDM with linkage analysis using a map with an average resolution of 10 cM. This approach has the advantage of assaying the entire genome. The alternative candidate-gene approach is attractive because of its low cost. However, an approach based solely on candidate genes is unlikely to detect all or even most NIDDM susceptibility loci, because many such genes may be novel with no presently known homologs.

Once a candidate region is identified by linkage analysis, association analysis to identify linkage disequilibrium may help narrow the region of interest. At that stage, identification of candidate genes within the region may prove useful, an approach that has been called positional candidate cloning (62).

Approaches to maximize power to detect linkage

We have taken several steps intended to maximize power to detect NIDDM genes. First, we have restricted family ascertainment to probands with the relatively early age of diagnosis of clinical diabetes of 35–60 years, because NIDDM is more strongly familial in relatives of early-onset probands (18) and environmental factors are likely to have greater effect at older ages.

Second, we have chosen a sampling design and analytic approach based on ASPs. Compared with the alternative of extended pedigrees, ASPs are easier to ascertain. Furthermore, analysis of ASPs does not require ad hoc specification of mode of inheritance. Instead, allele sharing between affected siblings is compared with expectations assuming no linkage, so that excess allele sharing suggests linkage.

Third, we have sought to minimize the number of families in which both parents are affected with NIDDM. Although such bilineal families can provide linkage information, for most genetic models, linkage information is substantially less than that provided by families with no affected parents or with one affected parent (63). To minimize bilineality, we gave preference to families with living parents and to those families in which one or both parents had lived a long life without known diabetes. Despite these efforts, it is likely that some of our families are bilineal, because only 42 parents in 577 families were available for a study visit, and because in one-sixth of the FUSION study families, neither parent had lived past the age of 61 years without known diabetes.

Fourth, we sought to avoid families with IDDM. Our decision was based on a wish to minimize heterogeneity of the clinical phenotype. During the natural course of NIDDM, the endogenous insulin secretion diminishes, often leading to a requirement for exogenous insulin (64,65). This situation can make it difficult to distinguish NIDDM from adult-onset IDDM, especially with the current trend in NIDDM therapy toward more aggressive treatment with insulin (66). The magnitude of the problem is suggested by results from the Second National Health and Nutrition Examination Survey (NHANES II), in which 7.4% of all diabetic patients were classified as having adult-onset IDDM (67). We have carefully phenotyped affected individuals to identify those with likely IDDM. We used a low serum C-peptide level as a marker for low endogenous insulin secretion, and antibodies to GAD as a marker for immune process, suggesting probable adult-onset IDDM. While other markers such as ICA and ICA512 antibodies may be less prevalent in older-onset IDDM, GAD antibody prevalence remains high in this age-group (68). Together with thorough medical record review, we classified 43 affected individuals in 39 FUSION families as probable adult-onset IDDM. These families will be excluded from most analyses.

Fifth, we have carried out extensive phenotyping in the families. Many cases of NIDDM are asymptomatic and can only be detected by testing glucose tolerance. In Finland, the proportion of such undetected cases of all NIDDM patients is estimated to be 25–40% (69,70). Thus, as much as possible, family members of NIDDM patients were tested for glucose tolerance to confirm their glucose tolerance status. In addition, we carried out FSIGTs together with Minimal Model analysis (22,71) in the unaffected offspring and spouses of the index case or of an affected sibling in a subset of our families. This approach estimates the individual physiological functions that determine the ability of the human organism to dispose of carbohydrate nutrients, including insulin sensitivity, glucose effectiveness, and acute insulin response. Knowledge of these phenotypes provides the opportunity to separately map the responsible genes for each component of the glucose homeostatic system. Perhaps even more important is that it will provide information to help assess the roles of the genes localized by linkage analysis of NIDDM.

In our study, we found that the nonaffected offspring of NIDDM patients were more insulin sensitive than nonaffected spouses of the patients. There are several factors that may explain this finding. *1*) Insulin sensitivity may decline with age (72), although not all studies show the association between biological age and insulin resistance when body weight and distribution of body fat has been taken into account (73,74). In the study by Chen et al. (72), 57- to 82-year-old men had 63% lower insulin sensitivity than 18- to 36-year-old men matched for body weight and fasting glucose. In our study, male spouses had 37% lower insulin sensitivity, and female spouses 19% lower insulin sensitivity, than the offspring. *2*) Obesity, abdominal obesity, and hyperglycemia are all well-known factors associated with insulin resistance (73,75). The spouses in our study were more obese than the offspring. Also, waist-to-hip ratios were higher, indicating a higher degree of

abdominal obesity in spouses. Moreover, spouses had slightly higher glucose values in the OGTT than their offspring. *3*) Of the spouses, 25% reported diabetes in their parents, suggesting a genetic predisposition to diabetes in some of the nonaffected spouses, with the proportion being approximately the same as expected in the Finnish general population (49,69,70).

Sixth, we have sampled a large number of ASP families. Our power calculations suggest that even 400 ASPs typed on a 10-cM map provide 82% power to detect a gene that confers a 1.8-fold excess risk to siblings of affected individuals at an LOD score of 3.0 (32). Even for a 1.4-fold excess risk, there is 81% power to detect genes at an LOD score of 1.0. Our large sample should also provide an excellent basis for carrying out confirmation studies for linkage or association results reported by other groups.

Limitations of the sampling design

Several of the choices we made to maximize power to detect NIDDM genes may have limited the set of NIDDM genes that we will be able to detect. By choosing a relatively homogeneous population such as the Finns, we may have limited the number of segregating genes available for detection. By selecting early diagnosed probands primarily from the NHDR, we are preferentially sampling more severely affected individuals. If the genes for early, more severe NIDDM are different from those for later, less severe disease, we will have increased our power to detect one set of genes at the expense of the other. We believe that increased power to identify a subset of NIDDM genes more than offsets loss of the theoretical opportunity to detect all such genes. By requiring the presence of an ASP, we might be identifying genes for familial NIDDM rather than for all NIDDM. However, given the high degree of familiality of NIDDM in all populations that have been studied, this problem does not appear to be significant, particularly because genes for familial disease often turn out to have a more general importance (76–78).

Several of these choices also made it more difficult to identify and recruit acceptable families. In Finland, only 3% of symptomatic NIDDM patients have their disease onset before the age of 40 years, and only 37% have it before the age of 60 years (64,79). Our requirement for a second affected sibling and zero or only one parent known to be affected, and our exclusion of families with IDDM, further restricted the pool of suitable families. Thus, screening more than 22,000 NIDDM patients resulted in only about 1,550 individuals (7%) meeting our study criteria. Even a lower proportion of suitable families was reported from the English study of Cook et al. (80), in which primary screening criteria were less stringent than ours. Their conclusion was to initiate large-scale collaborative multicenter studies to collect the family material necessary for the study of susceptibility genes for NIDDM. In contrast, the FUSION study has demonstrated that it is feasible, although not easy, to collect a large number of ASP families with NIDDM within one population even after applying careful selection criteria.

Future directions

Given our success in family collection and phenotypic analysis in the FUSION study, we are moving forward with FUSION 2, in which we hope both to extend our current FUSION families and to obtain additional families. In all our current FUSION families, we will type unaffected siblings to allow more accurate inference of identity by descent in the ASPs for purposes of linkage analysis. In our current FUSION nuclear families, we will sample a spouse and offspring of one of the affected siblings for purposes of haplotype determination and linkage disequilibrium mapping. We also plan to identify and sample a second set of Finnish families as a replication set to allow us to immediately test interesting chromosomal regions in a second, comparable set of families. In addition, we will obtain a sample of parent-offspring trios, in which the offspring is affected with NIDDM and the parents are living, to allow family-based association studies (81).

Even given a large initial sample of families and a second replication sample, detection of NIDDM susceptibility genes by linkage analysis will depend on a combination of hard work and good luck. Most critical will be the magnitude of the effect of the various susceptibility genes on disease risk. With a combined sample of perhaps 800 ASPs, we would have 69% power to detect a locus with the impact estimated by Hanis et al. (38) for their chromosome 2q locus at an LOD score of 3.0. However, for a locus that confers a 1.2-fold excess risk to siblings, even 800 ASPs would be expected to provide an LOD score of only 1.59 (32). Thus, in genetic studies of NIDDM and other common, complex genetic diseases, sharing results with other workers in the field and combining data from comparable samples will be critical to our success in identifying disease susceptibility genes.

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