

## Parent-Offspring Trios

# A Resource to Facilitate the Identification of Type 2 Diabetes Genes

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**The transmission disequilibrium test with use of trios (an affected proband with both parents) is a robust method for assessing the role of gene variants in disease that avoids the problem of population stratification that may confound conventional case/control studies and allows the detection of parent-of-origin effects. Trios have played a major role in defining genes in a number of polygenic conditions, including type 1 diabetes. We assessed the prevalence, clinical characteristics, and suitability for defining type 2 susceptibility genes of European type 2 diabetes trios. In a Caucasian population in the U.K., only 2.5% of type 2 patients had both parents alive. Using a nationwide strategy, we collected 182 trios defined by strict clinical criteria. Immunological and genetic testing resulted in the exclusion of 25 trios as a result of latent autoimmune diabetes ( $n = 13$ ), inconsistent family relationships ( $n = 7$ ), and maternally inherited diabetes and deafness ( $n = 5$ ). The 157 remaining probands had similar treatment requirements to familial type 2 diabetic subjects but presented at a younger age, were more obese, and more frequently had affected parents. Using this resource, we have not found any evidence for linkage disequilibrium between type 2 diabetes and the glucokinase gene markers GCK1 and GCK2 and the chromosome 20 marker D20S197. We conclude that European type 2 diabetes trios are difficult to collect but provide an important additional approach to dissecting the genetics of type 2 diabetes. *Diabetes* 48:2475–2479, 1999**

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ANOVA, analysis of variance; GCK, glucokinase gene; HNF, hepatocyte nuclear factor; LADA, latent autoimmune diabetes; MIDD, maternally inherited diabetes and deafness; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction; TDT, transmission disequilibrium test.

**L**inkage disequilibrium methods are assuming an increasingly important role in the genetic dissection of complex traits. They facilitate the evaluation of candidate polymorphisms (1), the fine mapping of linked regions, and potentially provide a method for genome-wide association studies (2). For detecting linkage disequilibrium, family-based association studies are preferred, because they avoid the problem of population stratification that may confound case/control studies (3). The transmission disequilibrium test (TDT) with the use of trios (an affected proband and both of his or her parents) has been successfully used in a number of polygenic conditions, including type 1 diabetes, to define susceptibility genes (1,4) and has allowed the detection of parent-of-origin effects (5). When available, therefore, parent-offspring trios are an excellent resource for detecting linkage disequilibrium as long as the subjects with living parents are representative of other subjects with the same disease. No large collection of European trios has been described in type 2 diabetes. The aim of our study was to assess the prevalence, clinical characteristics, and suitability for the detection of type 2 diabetes genes of a large European type 2 diabetes parent-offspring trios collection.

The prevalence of trios was estimated using 5,809 responses to a questionnaire-based population survey of family history in patients with diabetes (>99% U.K. Caucasian) in East Devon, U.K. (Table 1). We found that 2.5% of patients with a clinical diagnosis of type 2 diabetes had two living parents.

We obtained DNA and clinical characteristics from 182 type 2 diabetic trios by establishing a nationwide collection (the British Diabetic Association Warren 2 Trios) run from three U.K. centers (Exeter, Newcastle, and St. Mary's Hospital in London). Recruitment of patients was principally from hospital diabetes clinics, general practitioners, and the questionnaire survey. Subjects were considered suitable for the trios collection if the proband had type 2 diabetes and had two living parents and four grandparents of European descent (>98% were of U.K./Irish origin). Strict clinical criteria, as described in RESEARCH DESIGN AND METHODS, were used to reduce the admixture of subjects with type 1 diabetes and monogenic diabetes. To avoid inadvertently recruiting subjects with type 1 diabetes, recruitment was limited to trios for whom the proband was diagnosed after the age of 25 years,

TABLE 1

Potential availability of trios (diabetic proband with two living parents) and affected sibling pairs in a population-based questionnaire survey of 5,809 diabetic subjects (>99% U.K. Caucasian)

	Treatment category				
	Type 1	Type 2			
		All	Insulin	OHA	Diet
<i>n</i>	996	4,813	634	2,804	1,375
Both parents alive (%)	36.4	2.5	2.4	1.9	3.7
Living diabetic sibling (%)	12.0	15.2	17.5	15.3	13.8

OHA, oral hypoglycemic agents.

had been treated without insulin for at least 1 year after diagnosis, and did not have a family history of type 1 diabetes. Potential maturity-onset diabetes of the young (MODY) families were excluded if there were two generations with an autosomal dominant history of diabetes and at least one family member diagnosed before the age of 25 years. Maternally inherited diabetes and deafness (MIDD) was excluded in those instances in which there was maternal transmission of diabetes with deafness. It was possible to include only 53% of the subjects identified in the questionnaire survey in the final collection. This would suggest that the recruitment of 182 trios required review of ~15,000 type 2 diabetic subjects; this represents ~1% of all known type 2 diabetic subjects in the U.K.

To assess the suitability of the 182 trios for genetic analyses for type 2 diabetes, we performed genetic and immunological laboratory tests (Fig. 1). We identified seven (3.8%) families with inconsistent relationships by genotyping five microsatellite markers (total heterozygosity, 99.7%). GAD antibodies were measured in all probands. In 13 (8%), GAD antibodies were raised (>10 U), and these subjects were thinner (BMI 25.7 vs. 32.0 kg/m<sup>2</sup>, *P* = 0.0016) and more likely to be treated with insulin (69 vs. 15%, *P* < 0.0001) than the probands in whom GAD was not raised (Table 2). The mitochondrial 3243 tRNA<sup>Leucine</sup> mutation (mt3243) was found in five probands and their mothers at low levels of heteroplasmy (1–8%). These probands did not differ significantly from the other subjects

TABLE 2

Clinical characteristics of the 157 type 2 trios probands compared with subjects excluded as LADA, familial type 2 diabetic subjects, and a population survey of type 2 diabetic subjects

	Type 2 trios probands	LADA probands	<i>P</i>	Familial (sib-pair) type 2 probands	<i>P</i>	Population survey type 2 probands	<i>P</i>
<i>n</i> (M/F)	157 (98/59)	13 (8/5)		823 (435/388)		4,811 (2,615/2,176)	
Age of diagnosis (years)	40.1 ± 7.1	37.3 ± 5.5	0.18	55.4 ± 8.6	<0.0001	61.0 ± 12.5	<0.0001
Diabetes duration (years)	4.1 (1.7–9.6)	5.0 (2.8–8.9)	0.41	6.5 (2.7–15.5)	<0.0001	7.2 (2.8–16.7)	<0.0001
BMI	32.0 ± 6.8	25.7 ± 5.5	0.0016	29.0 ± 5.3	<0.0001	NA	
Percentage treatment (diet/OHA/insulin)	21/64/15	8/23/69	<0.0001	18/66/16	0.70	13/58/13	0.07
Percentage with at least one diabetic parent (father/mother/both)	67 25/21/21	46 23/15/8	0.61	43 12/30/1	<0.0001	24 7/15/2	<0.0001

*P* values are pairwise comparisons with type 2 trios probands. Normally distributed continuous variables are given as means ± SD and were compared using a two-tailed *t* test. Diabetes duration was skew distributed and so is given as geometric mean with the standard deviation range in parentheses and was log transformed before analysis. The  $\chi^2$  test was used to compare frequencies of paternal and maternal diabetes and treatment modalities. OHA, oral hypoglycemic agents.

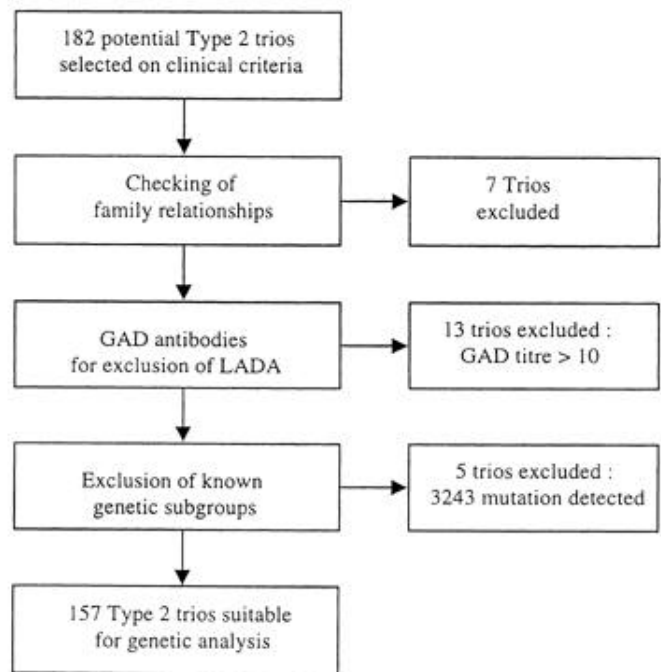


FIG. 1. Flow diagram of exclusion of trios after molecular genetic and GAD antibody testing.

and only two had a diabetic mother (data not shown). The most common U.K. MODY mutation, P291fsinsC in the hepatocyte nuclear factor (HNF)-1 $\alpha$  gene, which accounts for 20% of U.K. MODY (T.M.F., S.E., A.T.H, unpublished observations), was not detected in any of the probands. This finding, and the selection criteria used, suggest that MODY mutations will account for few of our trios probands, although we cannot exclude the presence of other MODY mutations in a small proportion of families.

The clinical characteristics of the 157 probands remaining after laboratory testing are shown in Table 2 and are compared with both U.K. Caucasian type 2 probands from the parallel British Diabetic Association Warren 2 sibling-pair study and with type 2 diabetic subjects from the population survey

TABLE 3

Power at  $P < 0.05$  (one-tailed) to detect linkage disequilibrium of 157 trios stratified by allele frequency, relative risk associated with allele, and whether associated allele acts as dominant or recessive

Allele frequency	Relative risk associated with allele					
	1.5		2.0		3.0	
	Dominant	Recessive	Dominant	Recessive	Dominant	Recessive
0.1	41	7	81	10	99.5	17
0.3	47	20	82	46	98.6	88
0.5	31	40	56	81	81	99.6

These estimates of power were calculated using the method of McGinnis (28).

(Table 2). All three groups of patients had similar treatment requirements. Trios probands were younger than the sibling-pair probands and the population sample having been diagnosed at an earlier age (40.1 vs. 55.4 vs. 61.0 years analysis of variance [ANOVA],  $P < 0.0001$ ) and having a shorter duration of diabetes (5.7 vs. 8.8 vs. 9.6 years ANOVA,  $P < 0.0001$ ). The trios probands more frequently had at least one affected parent (66.5 vs. 43.3 vs. 24.2%,  $P < 0.0001$ ) and were more obese than the sibling-pair probands (BMI 32.0 vs. 29.0 kg/m<sup>2</sup>,  $P < 0.0001$ ; not available for population survey). In the trios in which parental status could be directly tested, no maternal excess was seen, as reported in the two other series in which both parents were available for testing (6,7). In contrast, our reported data in the sibling pairs and population series showed an excess of maternal diabetes, in keeping with other studies that used reported data (8). There are many possible reasons for the differences between these differently ascertained series (9).

Table 3 shows the power of this collection of 157 trios to detect recessively and dominantly acting predisposing polymorphisms of different relative risk and different frequencies. Our collection of trios has good statistical power to confirm or refute many of the previously reported associations between candidate polymorphisms and type 2 diabetes but cannot be used when genes have a low relative risk ( $< 2.0$ ). We had 95% power at  $P = 0.01$  to detect the increased relative risk seen with the Z+2 allele of the glucokinase gene (GCK) 1 microsatellite in two previous studies (10,11), although this association was not seen in all data sets (12–14). Our cohort also has 82% power to replicate at the  $P = 0.01$  level the trans-

mission distortion seen at the microsatellite D20S197 at position 20q12-13.1 (15) in a predominantly Caucasian data set. However, this association was mainly among older type 2 diabetic patients (diagnosed after 47 years of age). Table 4 shows the results of TDT at GCK1, GCK2, and D20S197 in the trios collection. There was no evidence supporting preferential transmission of any of the common alleles ( $> 20\%$  parental chromosomes) of any of these three polymorphisms or the rare Z+2 GCK1 allele. These results do not confirm the previous positive associations at these loci and are in keeping with previous studies that have not found associations at these polymorphisms (12–14).

We have shown that with a coordinated nationwide strategy, it is possible to collect a large series of type 2 diabetes trios, even in a low-prevalence population such as European Caucasians. Previous studies have identified European Caucasian trios, but these have been small studies with insufficient power for genetic analysis (6,7). Our study has shown that in addition to strict clinical criteria, immunological and genetic testing is required to exclude latent autoimmune diabetes (LADA), inconsistent family relationships, and known monogenic subgroups of early-onset type 2 diabetes. Failure to exclude the 20 families as a result of this laboratory testing would have considerably reduced the power of this collection. The prevalence of LADA (8%) in this group and their clinical characteristics (nonobese, requiring insulin treatment) are consistent with those seen in other studies (16,17).

Are trios suitable for defining the genetic susceptibility to typical type 2 diabetes, despite being relatively rare? The presence of living parents could reflect early-onset disease and

TABLE 4

TDT analysis of GCK and the chromosome 20 marker D20S197 alleles

Marker	Allele (bp)	Frequency	Transmitted	Not transmitted	Transmission (%)	$P$
D20S197	191	0.25	61	57	52	0.71
	195*	0.27	60	60	50	1.00
GCK1	195 (Z)*	0.68	52	65	44	0.23
	197 (Z+2)*	0.05	16	11	59	0.34
	199 (Z+4)*	0.27	54	49	52	0.62
GCK2	137	0.67	55	55	50	1.00
	139	0.24	48	47	51	0.92

Allele size in base pairs is given. Only alleles  $> 20\%$  frequency in the parental population were included for analysis, except for GCK1 allele 197 bp, which has been variably associated with type 2 diabetes. A  $\chi^2$  test against expected 50:50 transmission was used to calculate probability ( $P$ ) of observed transmissions from heterozygous parents. The most common GCK1 allele has previously been reported as Z with other alleles as Z+2, Z+4, etc. \*Previously associated with type 2 diabetes.

hence younger parents, a more benign clinical course in the whole family or a different disease process, or a combination of these factors. The trios probands are diagnosed considerably younger than our familial type 2 diabetic and population-based comparison groups. Our data suggest that they have a similar disease process, with the early onset resulting from their increased obesity and the higher prevalence of parental diabetes resulting in an increased "gene dose," as previously suggested by O'Rahilly et al. (18). Trios probands are likely, therefore, to have a higher incidence of predisposing genes to both diabetes and obesity than "typical" type 2 diabetes found in the general population. The initial identification of susceptibility genes may therefore be easier in trios, and it should be possible to detect associations previously seen in unselected type 2 diabetic case/control studies if they do not result from population stratification. However, it does mean that the generalizability of any significant transmission distortion in trios will be known only when large case/control studies involving a population-based sample of type 2 diabetic subjects are used. This applies also to the affected sibling-pair design, which our study shows has a considerably higher prevalence of parental diabetes than our population sample. The initial identification of predisposing genes in populations selected to have a high incidence of predisposing genes with subsequent assessment in population samples is a logical and economical use of resources.

We believe that this collection of trios will prove a useful additional resource for the identification of susceptibility genes in Caucasian type 2 diabetic subjects. The use of the TDT in trios is a powerful design for familial association studies, which avoid the false-positive results that can occur in case/control studies as a result of population stratification. Our power calculations show that our collection has sufficient power to examine many polymorphisms that have been reported as showing association with type 2 diabetes in case/control studies, e.g., the Z+2 allele at GCK1 (10,11). Trios offer a resource in which parent-of-origin effects can be detected using appropriate analysis methods (19). However, differences in transmission seen between mothers and fathers may not result from imprinting of the region in question, since such a difference can have many explanations, including a maternal effect and differences in parental survival (19). Alternative family association approaches that are robust against bias due to population stratification include sibling-control and single-parent methods. One-parent families may be an important additional resource, although they have reduced power compared with trios, and it has been suggested that the reconstruction of parental genotypes in the absence of one or both parents introduces biases (20,21). The loss of power is offset by the increased availability of such families, since the amount of genotyping performed is not limiting and recently novel statistical methods to eliminate bias have been developed, particularly when multiple siblings are available and some families have both parents living (22–25). With either trios or single-parent families, the use of control families with unaffected probands would allow the detection of generalized segregation distortion at a locus in the population, which is an important possible cause of distortion in the absence of linkage disequilibrium (1).

We conclude that collections of type 2 diabetes trios are possible and offer a valuable additional resource that will aid in the genetic dissection of this disorder.

## RESEARCH DESIGN AND METHODS

**Prevalence of type 2 diabetes trios.** To assess the prevalence of type 2 diabetes trios, we carried out a survey of diabetic subjects in the English county of Devon (>99% Caucasian). First, 72 general practices were contacted, and 69 of them agreed to participate in the study. Between February 1996 and March 1998, questionnaires were sent to 8,319 patients with diabetes, and 6,133 (73%) were returned. In 5,807 subjects, it was possible to define as probable type 1 or type 2 diabetes from the clinical data details. Characteristics of these subjects are shown in Table 1. Potential subjects with type 2 diabetes and both parents alive were contacted to see if they conformed to the strict criteria described below and if all three members of the trio were available and willing to take part in a type 2 diabetes trios collection.

**Clinical criteria.** Subjects were considered suitable for the trios collection if they met the following criteria: 1) The proband had type 2 diabetes as shown by a) diagnosis after 25 years; b) if on diet treatment alone: biochemical confirmation of diagnostic blood glucose measurement (present or historical) or an HbA<sub>1c</sub> level above the normal range was required; c) if on insulin treatment: type 1 diabetes was excluded clinically by there being at least 1 year between diagnosis and commencement of insulin treatment, no ketoacidosis, no substantial weight loss before diagnosis, and no family history of type 1 diabetes (siblings, parents, uncles, aunts, or first cousins); d) potential MODY families were excluded clinically if there were two generations with an autosomal history of diabetes and at least one family member diagnosed before 25 years of age; and e) MIDD was excluded when there was maternal transmission of diabetes with deafness. 2) Both parents were alive and prepared to take part in the study. Affection status of parents was not a selection criterion. 3) Four grandparents of European Caucasian descent (in our collection, 98% of the families were of full U.K./Irish origin, with the remainder having at least one non-British/non-Irish [but European] grandparent).

In those instances in which probands and their parents met these criteria, informed consent was obtained from all family members for extraction of genomic DNA and subsequent genetic analysis. Only complete trios (i.e., those with both parents) were used in this study. Details of age, sex, height, weight, waist-to-hip ratio, and, where appropriate, details about diabetes and its treatment were collected from all subjects.

**Establishment of family relationships.** Five microsatellite markers were analyzed to detect inconsistent family relationships and sample errors (e.g., nonpaternity, adopted offspring, and sample mix-ups). For each DNA sample, the microsatellites GCK1 (heterozygosity 50%, primers: F-tgttgctagctgtagctgaactcatg, R-cccacacaaactgcctgtatta), GCK2 (60%, primers: F-aacagatagcctcattctg, R-tgtctgcaactactctac), D20S119 (82%, primers: F-tttccagattaggggtgatg, R-ctgacacagtttcagatctctac), D20S197 (76%, primers: F-tcctgtgctcctgtttaagatca, R-cagtgcttctccttagatg), and ADA (74%, primers: F-ccagatcgccactctact, R-agatagcatagatagaga) were amplified (combined heterozygosity 99.7%). Polymerase chain reaction (PCR) cycling conditions were as follows: 15-min denaturation at 94°C, 35 cycles consisting of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, and a final extension step of 30 min at 72°C. Each 10- $\mu$ l reaction consisted of 40 ng DNA, 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 1.5 mmol/l MgCl<sub>2</sub>, 200  $\mu$ mol/l dNTPs, 2.5 pmol each primer, and 0.25 U Ampliqaq Gold Taq polymerase (Perkin-Elmer, Warrington, U.K.). The products from each of the five PCR reactions were pooled and 1.5  $\mu$ l added to 3  $\mu$ l of loading buffer consisting of 0.375  $\mu$ l Genescan500 Tamra size standard and 2.625  $\mu$ l of formamide/blue dextran. Samples were denatured at 90°C for 2 min and 1.0  $\mu$ l loaded onto a 36-cm 4% polyacrylamide denaturing gel on an ABI 377 (Perkin-Elmer). Electrophoresis was carried out for 1 h at 3,000 V. Alleles were detected by Genescan and Genotyper software (ABI-PerkinElmer).

**GAD autoantibody testing.** All samples were tested for the presence of antibodies to GAD in the laboratory of Professor Franco Bottazzo, Royal London Hospital, by means of previously described methods (26). On the basis of control population data, probands with anti-GAD titers >10 U (range 13–318) were excluded. **Exclusion of MODY and MIDD.** To further exclude possible MODY and MIDD, we carried out genetic analyses. DNA was extracted from peripheral blood using standard protocols. We used a previously described rapid-screening technique (27) to test probands for the P291fsinsC HNF-1 $\alpha$  insertion. Position 291 is the single most common site for MODY mutations in the U.K., accounting for ~20% of all strictly defined MODY families and ~35% of the mutations in the HNF-1 $\alpha$  gene (T.M.F., S.E., A.T.H., unpublished observations).

To assess the prevalence of the mitochondrial 3243 mutation, a region encompassing the mitochondrial tRNA Leu(UUR) gene was amplified by PCR with the following oligonucleotide primers: forward, nt 3200–3219 5'-tataccacacaccacacag; and reverse, nt 3353–3334 5'-gcatgataaggggtacaat. PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 8 min. To quantify the 3243 mutation, a final cycle was performed (5 min at 94°C, 2 min at 55°C, and 12 min at 72°C) after the addition of 5  $\mu$ Ci of [ $\alpha$ -<sup>32</sup>P]dCTP, 10 pmol of each primer, and 2.5 U of Taq

polymerase. Labeled products were ethanol precipitated and resuspended in an appropriate volume of deionized water before digestion with the restriction enzyme HaeIII. Digested fragments were electrophoresed through a 12% non-denaturing polyacrylamide gel, and the radioactive fragments were quantified with a Phosphorimager screen and appropriate software (Molecular Dynamics, Sunnyvale, CA).

**Assessment of trios probands' characteristics.** We compared the clinical characteristics of the trios probands with 823 probands from the parallel Warren 2 sibling-pairs study designed for nonparametric linkage analysis (M.M., A.T.H., M.W., G.H., R.C.T., S.O'Rahilly, unpublished observations). For this study, families with at least two siblings diagnosed with type 2 diabetes (affection status being determined with the same criteria as for the trios probands) between the ages of 35 and 70 years. Families in which both parents were known to have been diabetic were excluded as were those in which there was a very high prevalence of diabetes in the siblings. All four grandparents were required to be of U.K. or Irish origin. The population sample was taken from the questionnaire described above.

HbA<sub>1c</sub> was measured in all unaffected parents. Subjects were considered as having diabetes if the HbA<sub>1c</sub> was >6.0 (the upper limit of the normal range). This was confirmed by the subsequent measurement of fasting glucose or the performing of an oral glucose tolerance test in the majority of cases.

**Power calculations.** To estimate the power of this trios collection, the methodology of McGinnis (28) was used. Genotype relative risks of 1.5, 2.0, and 3.0, assuming a biallelic marker, were used to calculate the power at  $P = 0.05$  (one-sided) of 157 trios, for allele frequencies of 0.1, 0.3, and 0.5 (Table 3). The power to detect transmission distortion was also estimated from previous association studies, e.g., for the GCK Z+2 allele, combining results from two studies in which positive associations with type 2 diabetes were found (10,11) gave allele frequencies of 4.4% in control subjects and 15.7% in affected subjects, giving a relative risk of 3.6 and a power of 95% at  $P = 0.01$  one-sided.

**Statistical analysis.** The groups were compared by means of the Student's unpaired  $t$  test, except for treatment modalities, in which case the  $\chi^2$  test was used.  $P < 0.05$  was considered as being statistically significant.

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## REFERENCES

1. Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506-516, 1993
2. Risch N, Merikangas K: The future of genetic studies of complex human diseases. *Science* 273:1516-1517, 1996
3. Lander ES, Schork NJ: Genetic dissection of complex traits (Review). *Science* 265:2037-2048, 1994
4. Bennett ST, Lucassen AM, Gough SCL, Powell EE, Undlien DE, Pritchard LE, Merriman ME, Kawaguchi Y, Dronsfield MJ, Pociot F, Nerup J, Bouzekri N, Cambon-Thomsen A, Ronningen KS, Barnett AH, Bain SC, Todd JA: Susceptibility to human type 1 diabetes at *IDDM2* is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat Genet* 9:284-291, 1995
5. Waterworth DM, Bennett ST, Gharani N, McCarthy MI, Hague S, Batty S, Conway GS, White D, Todd JA, Franks S, Williamson R: Linkage association of insulin gene VNTR regulatory polymorphism with polycystic ovary syndrome. *Lancet* 349:986-990, 1997
6. Cook JT, Page RC, O'Rahilly S, Levy J, Holman R, Barrow B, Hattersley AT, Shaw AG, Wainscoat JS, Turner RC: Availability of type II diabetic families for detection of diabetes susceptibility genes. *Diabetes* 42:1536-1543, 1993
7. Mitchell BD, Kammerer CM, Reinhart LJ, Stern MP, MacCluer JW: Is there an excess in maternal transmission of NIDDM? *Diabetologia* 38:314-317, 1995
8. Alcolado JC, Thomas AW: Maternally inherited diabetes mellitus: the role of mitochondrial DNA defects (Review). *Diabet Med* 12:102-108, 1995
9. Cox NJ: Maternal component in NIDDM transmission: how large an effect? (Letter). *Diabetes* 43:166-168, 1994
10. Chiu KC, Province MA, Dowse GK, Zimmet PZ, Serjeantson S, Permutt MA: A genetic marker at the glucokinase gene locus for type 2 (non-insulin-dependent) diabetes mellitus in Mauritian Creoles. *Diabetologia* 35:632-638, 1992
11. McCarthy MI, Hitman GA, Hitchins M, Riikonen A, Stengard J, Nissinen A, Tuomilehto-Wolf E, Tuomilehto J: Glucokinase gene polymorphisms: a genetic marker for glucose intolerance in a cohort of elderly Finnish men. *Diabet Med* 11:198-204, 1994
12. Hattersley AT, Saker PJ, Cook JT, Stratton IM, Patel P, Permutt MA, Turner RC, Wainscoat JS: Microsatellite polymorphisms at the glucokinase locus: a population association study in Caucasian type 2 diabetic subjects. *Diabet Med* 10:694-698, 1993
13. Tanizawa Y, Chiu KC, Province MA, Morgan R, Owens DR, Rees A, Permutt MA: Two microsatellite repeat polymorphisms flanking opposite ends of the human glucokinase gene: use in haplotype analysis of Welsh Caucasians with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 36:409-413, 1993
14. Malecki MT, Antonellis A, Casey P, Ji L, Wantman M, Warram JH, Krolewski AS: Exclusion of the hepatocyte nuclear factor 4 $\alpha$  as a candidate gene for late-onset NIDDM linked with chromosome 20q. *Diabetes* 47:970-972, 1998
15. Ji L, Malecki M, Warram HJ, Yang Y, Rich SS, Krolewski AS: New susceptibility locus for NIDDM is localized to human chromosome 20q. *Diabetes* 46:876-881, 1997
16. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R, Groop LC: UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. *Lancet* 350:1288-1293, 1997
17. Tuomi T, Carlsson A, Li H, Isomaa B, Miettinen A, Nilsson A, Nissén M, Ehmström B, Forsén B, Snickars B, Lahti K, Forsblom C, Saloranta C, Taskinen M, Groop LC: Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. *Diabetes* 48:150-157, 1999
18. O'Rahilly S, Spivey RS, Holman RR, Nugent Z, Clark A, Turner RC: Type II diabetes of early onset: a distinct clinical and genetic syndrome? *Br Med J* 294:923-928, 1987
19. Weinberg CR: Methods for detection of parent-of-origin effects in genetic studies of case-parent triads. *Am J Hum Genet* 65:229-235, 1999
20. Curtis D: Use of siblings as controls in case-control association studies. *Ann Hum Genet* 61:319-333, 1997
21. Curtis D, Sham PC: A note on the application of the transmission disequilibrium test when a parent is missing. *Am J Hum Genet* 56:811-812, 1995
22. Schaid DJ, Li HZ: Genotype relative-risks and association tests for nuclear families with missing parental data. *Genet Epidemiol* 14:1113-1118, 1997
23. Knapp M: The transmission/disequilibrium test and parental-genotype reconstruction: the reconstruction-combined transmission/disequilibrium test. *Am J Hum Genet* 64:861-870, 1999
24. Weinberg CR: Allowing for missing parents in genetic studies of case-parent triads. *Am J Hum Genet* 64:1186-1193, 1999
25. Sun FZ, Flanders WD, Yang QH, Khoury MT: Transmission disequilibrium test TDT when only one parent is available: the 1-TDT. *Am J Epidemiol* 150:97-104, 1999
26. Petersen JS, Hejnaes KR, Moody A, Karlsen AE, Marshall MO, Hoier-Madsen M, Boel E, Michelsen BK, Dyrberg T: Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. *Diabetes* 43:459-467, 1994
27. Frayling TM, Bulman MP, Appleton M, Bain SC, Hattersley AT, Ellard S: A rapid screening method for hepatocyte nuclear factor 1 alpha: prevalence in maturity-onset diabetes of the young and late-onset non-insulin dependent diabetes. *Hum Genet* 101:351-354, 1997
28. McGinnis RE: Hidden linkage: a comparison of the affected sib pair (ASP) test and transmission/disequilibrium test (TDT). *Ann Hum Genet* 62:159-179, 1998