JCI The Journal of Clinical Investigation

# HLA-DQB1-defined genetic susceptibility, beta cell autoimmunity, and metabolic characteristics in familial and nonfamilial insulin-dependent diabetes mellitus. Childhood Diabetes in Finland (DiMe) Study Group.

R Veijola, … , H K Akerblom, M Knip

*J Clin Invest.* 1996;98(11):2489-2495. https://doi.org/10.1172/JCI119067.

**Research Article**

Familial aggregation of insulin-dependent diabetes mellitus (IDDM) is a common phenomenon, but the reasons behind it are poorly understood. To investigate whether there is heterogeneity between familial and nonfamilial forms of IDDM we compared genetic, immunological, and clinical characteristics of diabetic children with and without an affected first-degree relative in a population-based series of Finnish children with IDDM. The frequencies of HLA-DQB1 genotypes known to be associated with high (DQB1*0302/0201) or moderate (*0302/x) IDDM risk in the Finnish population were increased, while the proportions of DQB1 genotypes associated with low or decreased risk for IDDM were reduced in the 121 familial cases as compared with the 574 nonfamilial cases (32.7 vs. 21.3%, 41.3 vs. 35.9%, 18.3 vs. 31.4%, and 7.7 vs. 11.4%, respectively; P = 0.002). The frequencies and serum concentrations of islet cell antibodies, insulin autoantibodies, and antibodies to the 65-kD isoform of glutamic acid decarboxylase were similar at diagnosis in the familial and nonfamilial cases. The 31 first-affected cases in the multiple case families were younger at diagnosis than the nonfamilial cases (6.9 vs. 8.5 yr; P < 0.05). The 90 second-affected familial cases had less severe metabolic decompensation at diagnosis than either the first-affected familial or nonfamilial cases. In conclusion, familial aggregation of IDDM in Finland is at least partly explained by a higher frequency of IDDM […]



# HLA-DQB1–defined Genetic Susceptibility, Beta Cell Autoimmunity, and Metabolic Characteristics in Familial and Nonfamilial Insulin-dependent Diabetes Mellitus

**Riitta Veijola,* Helena Reijonen,‡ Paula Vähäsalo,* Emad Sabbah,* Petri Kulmala,* Jorma Ilonen,‡ Hans K. Åkerblom,§ Mikael Knip,* and the Childhood Diabetes in Finland (DiMe) Study Group**

**Department of Pediatrics, University of Oulu, 90220 Oulu, Finland; ‡Turku Immunology Centre and Department of Virology, University of Turku, 20520 Turku, Finland; and §The Children's Hospital, II Department of Pediatrics, University of Helsinki, 00290 Helsinki, Finland*

## Abstract

**Familial aggregation of insulin-dependent diabetes mellitus (IDDM) is a common phenomenon, but the reasons behind it are poorly understood. To investigate whether there is heterogeneity between familial and nonfamilial forms of IDDM we compared genetic, immunological, and clinical characteristics of diabetic children with and without an affected first-degree relative in a population-based series of Finnish children with IDDM. The frequencies of HLA-DQB1 genotypes known to be associated with high (DQB1-*0302/0201) or moderate (*0302/x) IDDM risk in the Finnish population were increased, while the proportions of DQB1 genotypes associated with low or decreased risk for IDDM were reduced in the 121 familial cases as compared with the 574 nonfamilial cases (32.7 vs. 21.3%, 41.3 vs. 35.9%, 18.3 vs. 31.4%, and 7.7 vs. 11.4%, respectively; $P = 0.002$). The frequencies and serum concentrations of islet cell antibodies, insulin autoantibodies, and antibodies to the 65-kD isoform of glutamic acid decarboxylase were similar at diagnosis in the familial and nonfamilial cases. The 31 first-affected cases in the multiple case families were younger at diagnosis than the nonfamilial cases (6.9 vs. 8.5 yr; $P < 0.05$). The 90 second-affected familial cases had less severe metabolic decompensation at diagnosis than either the first-affected familial or nonfamilial cases. In conclusion, familial aggregation of IDDM in Finland is at least partly explained by a higher frequency of IDDM susceptibility genes in families with multiple affected individuals. The lack of differences in autoantibody levels between the familial and nonfamilial cases indicates homogeneity rather than heterogeneity in the pathogenetic process of beta cell destruction. (*J. Clin. Invest.* 1996. 98:2489–2495.) Key words: genetic heterogeneity • autoantibodies • diabetic ketoacidosis • C-peptide • glycated hemoglobin A**

## Introduction

Familial aggregation of insulin-dependent diabetes mellitus (IDDM)[1] is a well-established phenomenon demonstrated in many epidemiological studies. The proportion of subjects with IDDM having one or more affected first-degree relatives has fluctuated considerably, however (1–5), probably due to small sample sizes, selection bias, short follow-up periods, variations in family size, and other methodological problems. More recently, population-based data have become available, and fairly consistent prevalence rates of 11–13% for the presence of an affected first-degree relative have been reported in subjects with IDDM at the time of diagnosis (6–8). The risk of IDDM in siblings of diabetic probands up to the age of 30 yr has been estimated to be ~ 6–7% (9–12), although a higher long-term risk, 9.6% up to the age of 60 yr, has been reported recently in Denmark (12). Approximately 4% of the parents of IDDM patients (6, 7, 10) and 5% of the offspring are affected by the disease (1, 13). Intriguingly, it has been observed that it is more often the father than the mother of a child with IDDM who is affected (7, 8, 14).

The reasons for these epidemiological observations are unknown, but it has been suggested that familial cases are genetically more susceptible to IDDM, and that as yet unidentified environmental risk factors have clustered in certain families with multiple affected individuals. However, there have been several studies that have failed to detect differences in the frequency of the high risk HLA phenotypes between IDDM cases with and without an affected family member (2, 3, 15–17).

Because of the above-mentioned methodological problems and varying definitions used for both familial and sporadic IDDM cases, most sets of previous results are difficult to compare. Clearly, population-based studies are needed to find out whether there is heterogeneity between familial and nonfamilial forms of IDDM.

## Methods

*Patient population.* The population-based, nationwide "Childhood Diabetes in Finland" (DiMe) Study was started in 1986 to investigate the role of genetic, immunological, and environmental factors in the development of IDDM. From the beginning of September 1986 to the end of April 1989, 801 index cases aged 14 yr or younger were diagnosed with IDDM and invited to participate together with their fami-

Address correspondence to Riitta Veijola, M.D., University of Oulu, Department of Pediatrics, Kajaanintie 52 A, 90220 Oulu, Finland. Phone: 358-8-3152011; FAX: 358-8-3155559.

*Received for publication 24 May 1996 and accepted in revised form 26 September 1996.*

J. Clin. Invest.

0021-9738/96/12/2489/07 $2.00
Volume 98, Number 11, December 1996, 2489–2495

1. *Abbreviations used in this paper:* CI, confidence interval; GADA, antibodies to the 65-kD isoform of glutamic acid decarboxylase; GHb, glycated hemoglobin A; IAA, insulin autoantibodies; ICA, islet cell antibodies; IDDM, insulin-dependent diabetes mellitus; IQR, interquartile range; JDF-U, Juvenile Diabetes Foundation unit; RU, relative unit; SDS, standard deviation score.

lies. These included 90 index cases (11.2%) who had at least one first-degree relative with IDDM at the time of diagnosis, 86 having one such family member and four cases (0.5%) two; two with an affected father and sibling, one with the mother and a sibling, and one with two siblings. Altogether there were 45 index cases (5.6%) who had a father with IDDM at the time of presentation and 21 cases (2.6%) with an affected mother. 28 out of a total of 1,064 biological siblings (2.6%) had IDDM at diagnosis of the index case (7). The mean age of the subjects at diagnosis was 8.4 yr (3.8; SD) (range 0.8–14.9 yr). Informed consent was obtained from the subjects and their parents. The ethical committees of all participating hospitals approved the study protocol.

Probands with at least one first-degree relative with IDDM at the end of follow-up were defined as familial cases. These cases were classified into subjects who had an affected sibling (multiplex cases) and those who had an affected parent (multigenerational cases). In addition, the familial cases were grouped according to the presence of an affected family member either at the time of diagnosis (second-affected familial cases) or at the end of the follow-up (first-affected familial cases). Probands who had at least one unaffected sibling but no first-degree relatives with IDDM were defined as nonfamilial cases.

*Study protocol.* At the time of the DiMe study, insulin treatment for children with newly diagnosed IDDM in Finland was always initiated in hospitals, and thus a pediatrician in each hospital and a nurse in charge of the care of children with diabetes were trained in the data and blood sample collection procedures required for the DiMe protocol. Basic family data and information on the occurrence of chronic diseases, including IDDM, were obtained by means of a questionnaire filled in by the parents and completed with an interview, if necessary. The presence of familial IDDM was also checked against the data of the National Central Drug Registry based on the approvals for free-of-charge medication for diabetes at the Social Insurance Institution.

Blood samples were taken before initiation of insulin treatment to measure blood glucose, capillary blood gases, glycated hemoglobin A1 or A1C (GHb), serum C-peptide, and IDDM-associated autoantibodies. Genomic DNA was extracted from EDTA blood samples. Daily insulin doses, expressed in international units per kilogram of body weight per 24 h (IU/kg), were recorded during the first 24 h of treatment and 6, 12, 18, and 24 mo after diagnosis. Serum C-peptide concentrations and blood GHb levels were also determined at these time points to evaluate residual endogenous insulin secretion and the degree of metabolic control, respectively. Diabetic ketoacidosis at diagnosis was defined as a capillary blood pH $< 7.30$.

*Laboratory procedures.* Blood glucose concentrations and capillary-blood gases were measured by routine laboratory methods. The participating hospitals used standard methods to determine blood GHb. To make the GHb results comparable, these were expressed as standard deviation scores (SDS) based on the reference range in nondiabetic subjects in each hospital. Random serum C-peptide concentrations were analyzed radioimmunologically with antiserum K6 using a commercial kit (Novo Research Institute, Bagsvaerd, Denmark). Antibody-bound proinsulin was separated from C-peptide with polyethylene glycol before the assay. The detection limit for serum C-peptide was 0.02 nmol/liter.

Serum levels of insulin autoantibodies (IAA) were determined with a competitive radiobinding assay modified from that described by Palmer et al. (18). Endogenous insulin was removed with acid charcoal before the assay, and free and bound insulin were separated after incubation with mono-$^{125}$I(Tyr A 14)-labeled human insulin (Novo Research Institute) in the absence or presence of an excess of unlabeled insulin. The cut-off limit for IAA positivity was 54 nU/ml (99th percentile in 105 nondiabetic children).

The presence of islet cell antibodies (ICA) was analyzed by a standard immunofluorescence method applied to sections of frozen human pancreas (blood group O). Fluorescein-conjugated rabbit anti–human IgG (Behringwerke AG, Marburg, Germany) was used to detect these antibodies. End point dilution titers were determined for samples that were positive for ICA and the results were expressed in Juvenile Diabetes Foundation units (JDF-U) relative to an international reference standard (19). The detection limit for ICA was 2.5 JDF-U. Our laboratory has participated in the international workshops on the standardization of ICA methods, in which its sensitivity was 100%, specificity 98%, validity 98%, and consistency 98% in the fourth round.

Antibodies to the 65-kD isoform of glutamic acid decarboxylase (GADA) were measured using a competitive radioligand assay recently described by Petersen et al. (20). The limit for GADA positivity was a specific binding of 6.5 relative units (RU), representing the 99th percentile in a series of 372 healthy control children. The disease sensitivity of the present assay was 80% and the specificity 94% based on the 101 samples included in the second international GAD antibody workshop (21).

HLA-DQB1 typing was performed by a previously described method based on time-resolved fluorescence (22). Four sequence-specific oligonucleotide probes were used to identify the following DQB1 alleles known to be significantly associated with either susceptibility to or protection against IDDM in the Finnish population: DQB1*0302, DQB1*0201, DQB1*0602 or 0603, and DQB1*0301 (23). The recently described simplified classification of DQB1 genotypes into high risk (DQB1*0302/0201), moderate risk (DQB1*0302/x; x means 0302 or a nondefined allele), low risk (DQB1*0301/0302, DQB1*0201/0301, DQB1*0201/x, DQB1*0302/0602-3; x means 0201 or a nondefined allele), and decreased risk (DQB1*x/x, DQB1*0301/x, DQB1*0201/0602-3, DQB1*0301/0602-3, DQB1*0602-3/x; x means a nondefined allele) genotypes was used (23). HLA-DQB1 typing data were available for 661 index cases (82.5%).

*Statistical methods.* The results were evaluated statistically by cross-tabulation and $\chi^2$ statistics for classified variables, Student's $t$ test or one-way ANOVA for comparisons of normally distributed continuous variables, and the Mann-Whitney U test or Kruskall-Wallis ANOVA in the case of skewed distributions. Covariance analysis with adjustment for age was used when appropriate, and logarithmic transformations for variables with a skewed distribution were performed before this analysis. All the analyses were performed using the SPSS software package (SPSS Inc., Chicago, IL).

## Results

The median follow-up time for the families up to the end of October 1995 was 7.7 yr (range 6.5–9.2 yr). The number of biological siblings had increased by 104 in this time, representing children born into the families after the diagnosis of the index case. Altogether 45 additional first-degree relatives, comprising 43 siblings and two fathers in 40 families, had developed IDDM during the study. Thus the total number of families with multiple IDDM cases was 121 (15.1%) at the end of the follow-up, the frequencies of affected fathers and mothers being 5.9 and 2.6%, respectively, and the proportion of the siblings affected 6.1% (71/1,168).

About half of these 121 familial cases were multiplex cases ($n = 62$) and the others were multigenerational cases ($n = 59$). Nine probands having both a parent and a sibling with IDDM were included among the multiplex cases. The familial cases included 31 first-affected probands and 90 second-affected ones. After exclusion of the index cases with no biological siblings, a series of 574 nonfamilial cases remained. The classification of the index cases has been described in Fig. 1.

The basic epidemiological characteristics, distribution of genetic markers, occurrence of IDDM-associated autoantibodies, and degree of metabolic decompensation at diagnosis were compared between the 121 familial and 574 nonfamilial IDDM cases. No difference was observed in the seasonal inci-

*Table I. Clinical, Genetic, and Immunological Characteristics of Familial and Nonfamilial IDDM Cases at Diagnosis*

| | Familial cases | | | |
|---|---|---|---|---|
| | Multiplex $n = 62$ | Multigenerational $n = 59$ | Nonfamilial cases $n = 574$ | Cases with no biological siblings $n = 106$ |
| Clinical characteristics | | | | |
| Sex (male/female) | 34/28 | 26/33 | 327/247 | 53/53 |
| Age, yr (95% CI) | 7.7 (6.8–8.6) | 8.3 (7.3–9.3) | 8.5 (8.2–8.8) | 8.4 (7.7–9.2) |
| HLA-DQB1 genotypes[‡§‖] | | | | |
| High risk, *n* (%) | 22 (37.9) | 12 (26.1) | 101 (21.3) | 25 (30.1) |
| Moderate risk, *n* (%) | 21 (36.2) | 22 (47.8) | 170 (35.9) | 32 (38.6) |
| Low risk, *n* (%) | 11 (19.0) | 8 (17.4) | 149 (31.4) | 19 (22.9) |
| Decreased risk, *n* (%) | 4 (6.9) | 4 (8.7) | 54 (11.4) | 7 (8.4) |
| Serum autoantibodies | | | | |
| ICA, % (95% CI) | 83.6 (74.3–92.9) | 81.0 (70.9–91.1) | 84.4 (81.4–87.4) | 84.6 (77.7–91.5) |
| ICA, JDF-U (IQR) | 34 (9–160) | 36 (9–88) | 36 (10–141) | 36 (13–129) |
| IAA, % (95% CI) | 50.8 (38.1–63.6) | 43.9 (31.0–56.7) | 49.8 (45.6–54.0) | 44.6 (34.9–54.3) |
| IAA, nU/ml (IQR) | 57 (30–156) | 47 (24–137) | 54 (28–146) | 43 (14–125) |
| GADA, % (95% CI) | 72.4 (60.9–83.9) | 74.1 (62.8–85.4) | 73.1 (69.4–76.8) | 77.7 (69.7–85.7) |
| GADA, RU (IQR) | 15.4 (4.9–32.5) | 16.7 (6.4–45.4) | 21.3 (5.8–65.2) | 32.4 (8.0–90.9) |

Multiplex cases have a sibling with IDDM, multigenerational cases have an affected parent, and nonfamilial cases are subjects with at least one sibling but no first-degree relatives with IDDM. A simplified classification into high risk (DQB1*0302/0201), moderate risk (DQB1*0302/x; x means 0302 or a nondefined allele), low risk (DQB1*0301/0302, 0201/0301, 0201/x, 0302/0602-3; x means 0201 or a nondefined allele), and decreased risk (DQB1*x/x, 0301/x, 0201/0602-3, 0301/0602-3, 0602-3/x; x means a nondefined allele) DQB1 genotypes was used. Values are frequencies (95% CI), means (95% CI), or medians (IQR). [‡] $P = 0.004$ for comparison of DQB1 genotype distribution between multiplex and nonfamilial cases; [§] $P = 0.09$ for comparison of DQB1 genotype distribution between multigenerational and nonfamilial cases; [‖] DQB1 typing data available in 58 multiplex cases, 46 multigenerational cases, 474 nonfamilial cases, and 83 cases with no biological siblings.

dence of IDDM between these groups, both showing the lowest incidence in summer. Neither were any significant differences observed in the sex distribution or the mean age at diagnosis between the familial and nonfamilial cases. Subdivision of the 121 familial subjects into 62 multiplex and 59 multigenerational cases revealed no significant differences in age at diagnosis (Table I), whereas the 31 first-affected familial cases were found to be significantly younger at diagnosis than the nonfamilial cases (Table II).

The distribution of high, moderate, low, and decreased risk HLA-DQB1 genotypes differed significantly between the familial and nonfamilial cases ($P = 0.002$, Fig. 2). Considering single DQB1 genotypes the frequency of the DQB1*0302/0201 genotype was increased among the familial cases (32.7 vs. 21.3% in nonfamilial cases; $P = 0.02$). No differences were observed in the frequencies of other DQB1 genotypes (Table III), but the proportion of DQB1 genotypes other than DQB1*0302/0201 or DQB1*0302/x was significantly reduced among the familial cases (26.0 vs. 42.8% in nonfamilial cases; $P = 0.002$). The frequency of the DQB1*0302 allele tended to be increased among the familial IDDM cases (78.8 vs. 69.2%), $P = 0.065$; while the presence of the DQB1*0602, 0603, or 0301 protective alleles was decreased (12.5 vs. 22.8%; $P = 0.028$). The 62 multiplex cases differed significantly from the nonfamilial cases in the distribution of DQB1 genotypes, and a similar tendency was observed between the 59 multigenerational and nonfamilial cases, while no difference was found between the multiplex and multigenerational cases (Table I). Similarly, both the 31 first-affected cases and the 90 second-affected cases differed in DQB1 genotype distribution from the nonfamilial cases ($P = 0.06$ and 0.007; respectively), but no difference was observed between the first and second-affected cases (data not shown). There was no difference in the distribution of the four DQB1 defined risk genotypes between various age groups at the diagnosis of IDDM (Fig. 3).

No differences in either the prevalence or serum concentrations of ICA, IAA, or GADA at diagnosis were observed between the familial and nonfamilial cases. The frequencies and circulating levels of these autoantibodies in the nonfamilial, multiplex, and multigenerational IDDM cases are shown in Table I.

The 121 familial cases had lower blood glucose and GHb levels at diagnosis and a reduced frequency of ketoacidosis as compared with the nonfamilial cases {17.8 mmol/liter [interquartile range (IQR) 12.4–22.9 mmol/liter] vs. 20.8 mmol/liter [IQR 15.8–26.9 mmol/liter]; $P < 0.001$, 10.5 SDS [95% confidence interval (CI) 9.3–11.6] vs. 13.2 SDS [95% CI 12.6–13.8]; $P < 0.001$, and 9.1% [95% CI 3.7–14.5] vs. 25.5% [95% CI 21.8–29.2]; $P < 0.001$, respectively}. Higher serum C-peptide concentrations at diagnosis and lower insulin doses during the initial 24 h were also observed among the familial cases [0.19 nmol/liter (IQR 0.12–0.30) vs. 0.15 nmol/liter (IQR 0.10–0.24); $P = 0.008$ and 0.93 IU/kg (95% CI 0.81–1.07) vs. 1.25 IU/kg (95% CI 1.19–1.31); $P < 0.001$]. Subdivision of the familial cases into first- and second-affected ones showed that the 90 second-affected familial cases had lower blood glucose and GHb levels, a lower frequency of ketoacidosis, higher serum concentrations of C-peptide, and lower insulin dosage during the initial 24 h than the nonfamilial cases, whereas the 31 first-affected familial cases had the same degree of metabolic decompensation as the nonfamilial cases (Table II). No differences were observed between these groups in either serum

*Table II. Comparison of Metabolic Characteristics at Diagnosis and during the Initial 2 Years of IDDM between the Familial and Nonfamilial IDDM Cases*

| | Familial cases | | | |
|---|---|---|---|---|
| | First-affected | Second-affected | Nonfamilial cases | Cases with no biological siblings |
| | $n = 31$ | $n = 90$ | $n = 574$ | $n = 106$ |
| Data at diagnosis | | | | |
| Age, yr (95% CI) | 6.9 (5.6–8.2)* | 8.4 (7.6–9.2) | 8.5 (8.2–8.8) | 8.4 (7.7–9.2) |
| Blood glucose, mmol/liter (IQR) | 20.3 (15.2–25.3) | 16.7 (12.1–21.6)‡§ | 20.8 (15.8–26.9) | 19.0 (14.7–25.4) |
| GHb, SDS (95% CI) | 11.9 (9.7–14.2) | 10.1 (8.7–11.4)‡§ | 13.2 (12.6–13.8) | 14.1 (12.5–15.8) |
| Ketoacidosis, % (95% CI) | 21.4 (6.2–36.6) | 4.9 (0.2–9.6)‡§‖ | 25.5 (21.8–29.2)§ | 15.2 (8.1–22.3) |
| Serum C-peptide, nmol/liter (IQR) | 0.14 (0.10–0.24) | 0.21 (0.14–0.34)‡‖ | 0.15 (0.10–0.24) | 0.17 (0.12–0.26) |
| Insulin dose, IU/kg/24 h (95% CI) | 1.26 (0.92–1.65) | 0.82 (0.70–0.95)‡§‖ | 1.25 (1.19–1.31) | 1.12 (0.99–1.24) |
| Follow-up data | | | | |
| 6 mo | | | | |
| Serum C-peptide, nmol/liter (IQR) | 0.14 (0.07–0.32) | 0.19 (0.10–0.32) | 0.21 (0.11–0.33) | 0.23 (0.11–0.39) |
| Insulin dose, IU/kg (95% CI) | 0.41 (0.32–0.51) | 0.44 (0.39–0.49) | 0.45 (0.44–0.47) | 0.43 (0.39–0.48) |
| GHb, SDS (95% CI) | 4.1 (2.0–6.2) | 4.6 (3.8–5.4) | 4.0 (3.6–4.3) | 4.5 (3.6–5.4) |
| 12 mo | | | | |
| Serum C-peptide, nmol/liter (IQR) | 0.06 (0.04–0.14) | 0.11 (0.04–0.27) | 0.11 (0.04–0.21) | 0.12 (0.06–0.24) |
| Insulin dose, IU/kg (95% CI) | 0.59 (0.51–0.66) | 0.57 (0.51–0.62) | 0.58 (0.56–0.60) | 0.56 (0.52–0.61) |
| GHb, SDS (95% CI) | 3.8 (2.5–5.0)*§ | 6.9 (5.9–7.9)*‖ | 5.5 (5.1–5.9) | 5.7 (4.7–6.7) |
| 24 mo | | | | |
| Serum C-peptide, nmol/liter (IQR) | 0.03 (0.03–0.06) | 0.04 (0.03–0.12) | 0.04 (0.03–0.08) | 0.04 (0.00–0.07) |
| Insulin dose, IU/kg (95% CI) | 0.71 (0.62–0.80) | 0.71 (0.66–0.77) | 0.72 (0.70–0.74) | 0.73 (0.69–0.77) |
| GHb, SDS (95% CI) | 5.7 (4.4–6.9) | 8.2 (7.1–9.4)*‖ | 6.5 (6.0–6.9) | 7.4 (6.0–8.7) |

First-affected familial cases are defined as subjects who had no first-degree relatives with IDDM at the time of diagnosis, but a sibling or parent who progressed to IDDM during the follow-up period. Second-affected familial cases are subjects who had a first-degree relative with IDDM at the time of diagnosis and nonfamilial cases are subjects with at least one sibling but no first-degree relatives with IDDM. Values are frequencies (95% CI), means (95% CI), or medians IQR). *$P < 0.05$ vs. nonfamilial, ‡$P < 0.001$ vs. nonfamilial, ‖$P < 0.05$ vs. first-affected, §$P < 0.05$ vs. cases with no biological siblings.

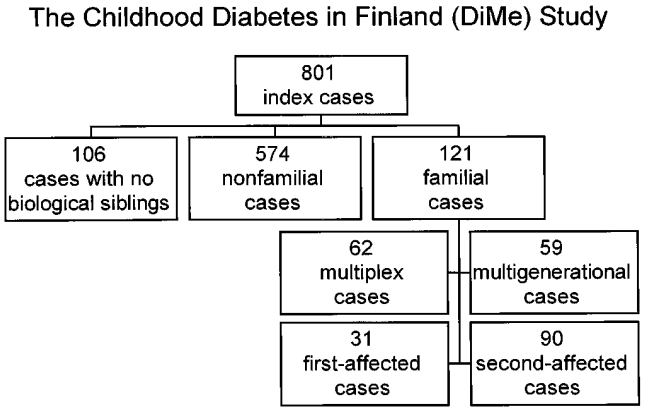


*Figure 1.* Classification of the index cases according to the presence of a first-degree relative with IDDM. Nonfamilial cases are subjects with at least one biological sibling but no first-degree relatives with IDDM. Familial cases are subjects with at least one first-degree relative with IDDM at the end of follow-up. Multiplex cases are subjects with an affected sibling. Multigenerational cases are subjects with an affected parent. First-affected cases are subjects who had no first-degree relatives with IDDM at the time of diagnosis but a sibling or parent who progressed to IDDM during the follow-up period. Second-affected cases are subjects who had a first-degree relative with IDDM at the time of diagnosis.

C-peptide concentrations or daily insulin doses during the initial 2 yr of IDDM, whereas GHb levels were significantly higher during the second year among the second-affected familial cases than in either of the other groups (Table II).

All the analyses were repeated after including the 106 index cases with no biological siblings in the group of nonfamilial cases, but this did not change the above-mentioned results. Neither were any differences observed in the results when the nine cases having both a parent and a sibling with IDDM were considered as multigenerational cases.

## Discussion

Opportunities to study the etiology of human IDDM are optimal in Finland because the incidence of IDDM is the highest in the world (24), the population has a culturally and genetically homogenous background, and health care for patients with diabetes is well organized. In the nationwide DiMe study the proportion of children with IDDM having an affected first-degree relative at the time of diagnosis was 11.2% (7), but after a median follow-up period of 7.7 yr, 15.1% of the index cases had a first-degree relative with the disease. These figures are in good accordance with the data of a recent Danish study reporting that 22.4% of subjects with childhood-onset IDDM

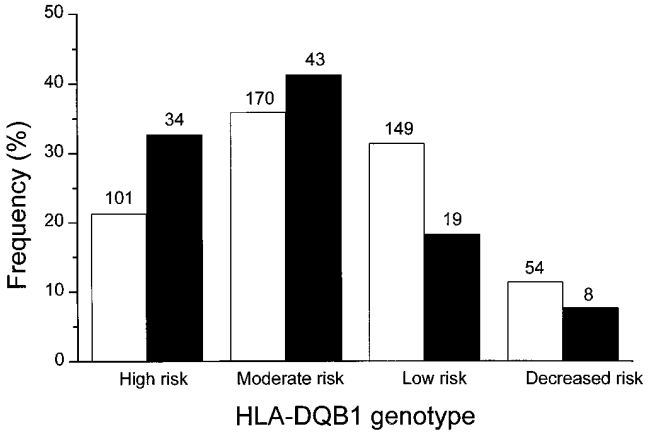


*Figure 2.* Distribution of HLA-DQB1 genotypes in familial (*filled bars*) and nonfamilial (*open bars*) IDDM cases. A simplified classification into high risk (DQB1*0302/0201), moderate risk (DQB1*0302/x; x means 0302 or a nondefined allele), low risk (DQB1*0301/0302, DQB1*0201/0301, DQB1*0201/x, DQB1*0302/0602-3; x means 0201 or a nondefined allele), and decreased risk (DQB1*x/x, DQB1*0301/x, DQB1*0201/0602-3, DQB1*0301/0602-3, DQB1*0602-3/x; x means a nondefined allele) DQB1 genotypes was used. Numbers of cases are shown above the columns. $P = 0.002$ in $\chi^2$ analysis between the two groups.

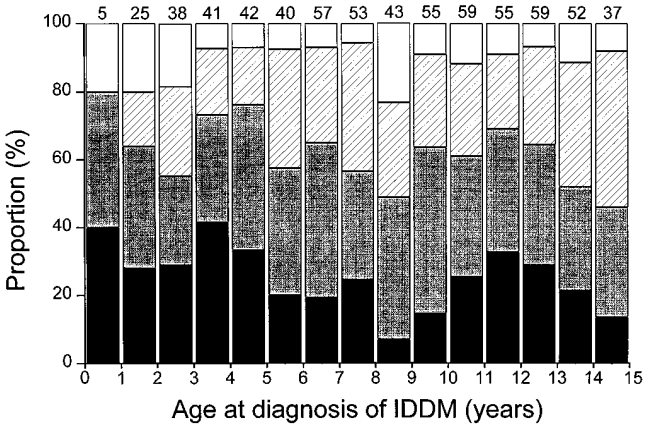


*Figure 3.* Proportion of subjects with high risk (*black bars*), moderate risk (*shaded bars*), low risk (*striped bars*), and decreased risk (*white bars*) HLA-DQB1 genotypes in various age groups defined at 1-yr intervals at the diagnosis of IDDM. Figures above the columns represent the number of subjects within each age group. $P = 0.06$ in the $\chi^2$ test.

and disease duration > 30 yr have a first-degree relative with IDDM (12).

The present investigation was aimed at searching for evidence of possible heterogeneity between familial and nonfamilial IDDM cases. A review of previous studies focusing on this topic reveals that varying definitions for both familial and nonfamilial forms of IDDM have been used, and therefore interpretation and comparison of these studies is complicated. We defined a nonfamilial or sporadic IDDM case as a diabetic proband with at least one biological sibling but no first-degree relatives having IDDM. Thus families with a single child with IDDM and both parents unaffected were excluded from the analysis, since they can be considered as potentially multiplex families.

*Table III. The Frequencies of Various DQB1 Genotypes and DQB1 Alleles in Familial and Nonfamilial IDDM Cases*

| DQB1 genotype or allele | Familial cases *n* (%) | Nonfamilial cases *n* (%) | $X^2$ statistics |
|---|---|---|---|
| 0302/0201 | 34 (32.7) | 101 (21.3) | $P = 0.02$ |
| 0302/x | 43 (41.3) | 170 (35.9) | NS |
| 0301/0302 | 3 (2.9) | 32 (6.8) | NS |
| 0201/0301 | 3 (2.9) | 26 (5.5) | NS |
| 0201/x | 11 (10.6) | 66 (13.9) | NS |
| 0302/602-3 | 2 (1.9) | 25 (5.3) | NS |
| x/x | 3 (2.9) | 29 (6.1) | NS |
| 0301/x | — | 15 (3.2) | NS |
| 0201/602-3 | 2 (1.9) | 4 (0.8) | NS |
| 0301/602-3 | 3 (2.9) | 2 (0.4) | NS |
| 602-3/x | — | 4 (0.8) | NS |
| 0302 | 82 (78.8) | 328 (69.2) | $P = 0.065$ |
| 0201 | 50 (48.1) | 197 (41.6) | NS |
| 0301 | 9 (8.7) | 75 (15.8) | $P = 0.09$ |
| 602-3 | 7 (6.7) | 35 (7.4) | NS |

DQB1 typing data were available for 104 familial cases and 474 nonfamilial cases.

The familial cases were slightly younger at diagnosis of IDDM, the difference being statistically significant when the 31 first-affected familial cases were compared with the nonfamilial cases (6.9 vs. 8.5 yr). These results are in line with those of a recent Danish study (8), which also reported a lower mean age at diagnosis in the first-affected children in multiple case families than in sporadic cases (7.6 vs. 8.6 yr). In that study the second-affected siblings were observed to be significantly older at diagnosis (11.2 yr) than the first-affected familial cases. Two reports from Pittsburgh quote an older age at diagnosis among second-affected cases than among nonfamilial cases (9, 17), whereas no difference in age at diagnosis was found between first-affected familial cases and nonfamilial cases (9). A recent Swedish study based on a large cohort of patients with childhood onset diabetes also reported an older age at diagnosis among second-affected siblings as compared with nonfamilial cases (9.0 vs. 8.3 yr) (25). The first-affected cases from multiple case families were not analyzed separately in that study. Our results indicated that the second-affected cases were of the same age at diagnosis as the nonfamilial cases, which may be due to the fact that we included also children with an affected parent in the group of second-affected cases. The varying results may be related also to differences in the incidence rates of IDDM between populations, and particularly to the observation of an increasing incidence of IDDM in most European countries but not consistently so in North America (24). Observations on high-incidence populations have also indicated that the increase in incidence has been most marked in the youngest age groups (26–28).

The present results provide strong evidence for the concept that genetic factors are important determinants of the familial aggregation of IDDM in Finland. So far, this is the first study showing that the frequency of the DQB1*0302/0201 genotype found to be associated with enhanced IDDM risk is increased among affected subjects with a positive family history of

IDDM as compared with nonfamilial cases. Earlier, only Svejgaard et al. (29) had been able to show a significantly elevated frequency of the DR3/4 phenotype among familial cases, but the difference in the frequency of the DQB1*0302/0201 genotype between familial and sporadic IDDM cases in Denmark was not significant in a recent nationwide survey (42 vs. 34%, respectively) (16). However, the present series is the first population-based study in which the frequencies of various DQB1 genotypes have been compared between familial and nonfamilial cases, and our study population is also considerably larger than the Danish one. It may also be possible that there are differences between populations in genetic and/or environmental factors which explain the clustering of IDDM in families.

Previous studies have indicated that there are differences in the frequencies of high risk HLA class II genotypes between childhood and adult-onset IDDM cases, the proportion of DR3/DR4 heterozygotes being higher in subjects with young age at diagnosis (4, 30). Our study population included only childhood-onset cases, and when analyzing the distribution of the four DQB1-defined risk genotypes in different age groups no significant difference could be observed. Accordingly, the age at diagnosis can hardly induce any bias when comparing the frequencies of various DQB1 genotypes between familial and nonfamilial cases.

In this study no differences were found in the overall distribution of DQB1 genotypes between the multiplex and multigenerational or first-affected and second-affected familial cases, but the highest frequency of the DQB1*0302/0201 genotype was observed among the multiplex cases, while the DQB1*0302/x genotype was the most common one in the multigenerational cases (Table I). This observation could indicate that the affected offspring in the multigenerational cases have inherited the DQB1*0302-positive haplotype from the affected parent, while in multiplex cases it would be the DQB1*0302/0201 combination which is primarily responsible for the enhanced IDDM risk.

Although our results show that affected subjects in multiple case families are genetically more susceptible to IDDM, the role of environmental factors in the familial aggregation of IDDM cannot be ruled out. In fact, it is likely that as yet unidentified genetic–environmental interactions are the most important elements explaining the development of IDDM in subjects from the same families. Some evidence has already been obtained for a possible link between certain enteroviruses and high risk HLA genotypes, by virtue of the observation that the frequency of antibodies to Coxsackie-B viruses was higher in newly diagnosed IDDM subjects with the HLA-DR4 than in those with other DR types (31, 32).

Various autoantibodies, like ICA, IAA, and GADA, have been used as immunological markers of the development of IDDM when aiming for prediction and prevention of IDDM. The lack of a difference in either the occurrence of these autoantibodies or their serum levels between familial and sporadic cases suggests that similar pathogenetic processes resulting in the appearance of various autoantibodies take place in the prediabetic period in subjects with or without a family history of IDDM. Accordingly, observations on the evolution of IDDM obtained mainly from first-degree relatives of subjects with the disease are likely to be applicable to sporadic cases, although there are differences in the predictive value of various IDDM-associated autoantibodies between families and the general population (33, 34).

The absence of any differences in the degree of metabolic decompensation at diagnosis between the first-affected familial and nonfamilial cases, whereas the second-affected familial cases had milder metabolic alterations, indicates that the favorable metabolic condition among those with a family history of IDDM is due to the increased awareness of the disease and its symptoms in the family and confirms the results reported previously by O'Leary et al. (17). The higher GHb levels in the second-affected familial cases observed during the second year of IDDM may be related to psychological factors reflected by a higher acceptance rate of hyperglycemia and fear of hypoglycemia as a consequence of previous experiences.

In conclusion, the increased frequency of DQB1*0302/0201 genotype found in familial IDDM cases partly explains the familial aggregation of the disease. No differences were observed either in the frequencies or serum levels of IDDM-associated autoantibodies between familial and nonfamilial cases, and the degree of metabolic decompensation was similar between the first-affected familial and nonfamilial cases. Consequently, a homogeneous rather than a heterogeneous pathogenetic process seems to be responsible for beta cell destruction in familial and nonfamilial IDDM.

## Acknowledgments

We thank Ms. Sirpa Anttila, Ms. Riitta Päkkilä, Ms. Päivi Ronkainen, and Ms. Susanna Heikkilä for their skillful technical assistance.

The Childhood Diabetes in Finland (DiMe) Study Group is composed of the following members. Principal investigators are H.K. Åkerblom and J. Tuomilehto. Coordinators are R. Lounamaa and L. Toivanen. Data managers are E. Virtala and J. Pitkäniemi. Local investigators are A. Fagerlund, M. Flittner, B. Gustafsson, C. Häggquist, A. Hakulinen, L. Herva, P. Hiltunen, T. Huhtamäki, N.-P. Huttunen, T. Huupponen, M. Hyttinen, T. Joki, R. Jokisalo, M.-L. Käär, S. Kallio, E.A. Kaprio, U. Kaski, M. Knip, L. Laine, J. Lappalainen, J. Mäenpää, A.-L. Mäkelä, K. Niemi, A. Niiranen, A. Nuuja, P. Ojajärvi, T. Otonkoski, K. Pihlajamäki, S. Pöntynen, J. Rajantie, J. Sankala, J. Schumacher, M. Sillanpää, M.-R. Ståhlberg, C.-H. Stråhlmann, T. Uotila, M. Väre, P. Varimo, and G. Wetterstrand. Special investigators are A. Aro, M. Hiltunen, H. Hurme, H. Hyöty, J. Ilonen, J. Karjalainen, M. Knip, P. Leinikki, A. Miettinen, T. Petäys, L. Räsänen, H. Reijonen, A. Reunanen, T. Saukkonen, E. Savilahti, E. Tuomilehto-Wolf, P. Vähäsalo, and S.M. Virtanen.

This study was supported by the Alma and K.A. Snellman Foundation (Oulu, Finland), the Emil Aaltonen Foundation (Tampere, Finland), the Foundation for Pediatric Research and the Ulla Hjelt Fund (Helsinki, Finland), Maud Kuistila Foundation (Helsinki, Finland), and the Medical Research Council, the Academy of Finland. The Childhood Diabetes in Finland project has been supported by grants from the National Institutes of Health (DK-37957), the Sigrid Jusélius Foundation, the Association of Finnish Life Insurance Companies, the University of Helsinki, the Novo Nordisk Foundation, and Novo Nordisk A/S (Bagsvaerd, Denmark).

## References

1. Degnbol, B., and A. Green. 1978. Diabetes mellitus among first- and second-degree relatives of early onset diabetics. *Ann. Hum. Genet.* 42:25–34.

2. Bengsch, N., J. Köbberling, G. Eckert, and B. Willms. 1978. HLA-typing in juvenile diabetics with and without positive family history and in families with one and two diabetic siblings. *Diabetologia.* 15:447–451.

3. Jaworski, M.A., E. Colle, R.D. Guttman, M.M. Belmonte, B. Taylor, M.P. Crepeau, J. Wilkins, and R. Poirier. 1980. Insulin dependent diabetes: a comparison of families with single and multiple affected siblings. *Diabetologia.* 19:97–100.

4. Karjalainen, J., P. Salmela, J. Ilonen, H.M. Surcel, and M. Knip. 1989. A comparison of childhood and adult type I diabetes mellitus. *N. Engl. J. Med.*

320:881–886.

5. Allen, C., M. Palta, and D.J. D'Alessio. 1991. Risk of diabetes in siblings and other relatives of IDDM subjects. *Diabetes.* 40:831–836.

6. Dahlquist, G., L. Blom, G. Holmgren, B. Hägglöf, Y. Larsson, G. Sterky, and S. Wall. 1985. The epidemiology of diabetes in Swedish children 0-14 years: a six-year prospective study. *Diabetologia.* 28:802–808.

7. Tuomilehto, J., R. Lounamaa, E. Tuomilehto-Wolf, A. Reunanen, E. Virtala, E.A. Kaprio, H.K. Åkerblom, and the Childhood Diabetes in Finland (DiMe) Study Group. 1992. Epidemiology of childhood diabetes mellitus in Finland: background of a nationwide study of type 1 (insulin-dependent) diabetes mellitus. *Diabetologia.* 35:70–76.

8. Pociot, F., K. Norgaard, N. Hobolth, O. Andersen, and J. Nerup. 1993. A nationwide population-based study of the familial aggregation of type-1 (insulin-dependent) diabetes-mellitus in Denmark. *Diabetologia.* 36:870–875.

9. Wagener, D.K., J.M. Sacks, R.E. LaPorte, and J.M. MacGregor. 1982. The Pittsburgh study of insulin-dependent diabetes mellitus. Risk for diabetes among relatives of IDDM. *Diabetes.* 31:136–144.

10. Tarn, A.C., J.M. Thomas, B.M. Dean, D. Ingram, G. Schwarz, G.F. Bottazzo, and E.A. Gale. 1988. Predicting insulin-dependent diabetes. *Lancet.* I: 845–850.

11. Gavard, J.A., J.S. Dorman, R.E. LaPorte, T.J. Orchard, A.L. Drash, M.M. Trucco, S.F. Kelsey, J.N. Kostraba, and D.J. Becker. 1992. Sex differences in secondary attack rate of IDDM to siblings of probands through older ages. *Diabetes Care.* 15:559–561.

12. Lorenzen, T., F. Pociot, P. Hougaard, and J. Nerup. 1994. Long-term risk of IDDM in first-degree relatives of patients with IDDM. *Diabetologia.* 37: 321–327.

13. Tillil, H., and J. Köbberling. 1987. Age-corrected empirical genetic risk estimates for first degree relatives of IDDM patients. *Diabetes.* 36:93–99.

14. Warram, J.H., A.S. Krolewski, M.S. Gottlieb, and C.R. Kahn. 1984. Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N. Engl. J. Med.* 311:149–152.

15. Anderson, C.E., S.E. Hodge, R. Rubin, J.I. Rotter, P.I. Terasaki, W.J. Irvine, and D.L. Rimoin. 1983. A search for heterogeneity in insulin dependent diabetes mellitus (IDDM): HLA and autoimmune studies in simplex, multiplex and multigenerational families. *Metab. Clin. Exp.* 32:471–477.

16. Pociot, F., K.S. Rønningen, R. Bergholdt, T. Lorenzen, J. Johannesen, K. Ye, C.A. Dinarello, J. Nerup, and the Danish Study Group of Diabetes in Childhood. 1994. Genetic susceptibility markers in Danish patients with type 1 (insulin-dependent) diabetes: evidence for polygenicity in man. *Autoimmunity.* 19:169–178.

17. O'Leary, L.A., J.S. Dorman, R.E. LaPorte, T.J. Orchard, D.J. Becker, L.H. Kuller, M.S. Eberhardt, D.E. Cavender, B.S. Rabin, and A.L. Drash. 1991. Familial and sporadic insulin-dependent diabetes: evidence for heterogeneous etiologies? *Diabetes Res. Clin. Pract.* 14:183–190.

18. Palmer, J.P., C.M. Asplin, P. Clemons, K. Lyen, O. Tatpati, P.K. Raghu, and T.L. Paquette. 1983. Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science (Wash. DC).* 222:1337–1339.

19. Lernmark, Å., J.L. Molenaar, W.A. van Beers, Y. Yamaguchi, S. Nagataki, J. Ludvigsson, and N.K. Maclaren. 1991. The fourth international serum exchange workshop to standardize cytoplasmic islet cell antibodies. *Diabetologia.* 34:534–535.

20. Petersen, J.S., K.R. Hejnaes, A. Moody, A.E. Karlsen, M.O. Marshall, M. Hoiermadsen, E. Boel, B.K. Michelsen, and T. Dyrberg. 1994. Detection of GAD(65) antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. *Diabetes.* 43:459–467.

21. Schmidli, R.S., P.G. Colman, E. Bonifacio, and participating laboratories. 1995. Second international GAD antibody workshop. *Diabetes.* 44:636–640.

22. Sjöroos, M., A. Iitiä, J. Ilonen, H. Reijonen, and T. Lövgren. 1995. Triple-label hybridization assay for type 1 diabetes-related HLA alleles. *BioTechniques.* 18:870–877.

23. Ilonen, J., H. Reijonen, E. Herva, M. Sjöroos, A. Iitiä, T. Lövgren, R. Veijola, M. Knip, H.K. Åkerblom, and the Childhood Diabetes in Finland (DiMe) Study Group. 1996. Rapid HLA-DQB1 genotyping for four alleles in the assessment of IDDM risk in the Finnish population. *Diabetes Care.* 19:795–800.

24. Karvonen, M., J. Tuomilehto, I. Libman, and R. LaPorte. 1993. A review of the recent epidemiological data on the worldwide incidence of type-1 (insulin-dependent) diabetes mellitus. *Diabetologia.* 36:883–892.

25. Dahlquist, G.G., and L.R. Mustonen. 1995. Clinical onset characteristics of familial versus nonfamilial cases in a large population-based cohort of childhood-onset diabetes patients. *Diabetes Care.* 18:852–854.

26. Hearnshaw, J.K. 1986. Childhood and after: a review of childhood diabetes in an English community 1930-1985. *In* Diabetes 1985. M. Serrano-Rios and P.J. Lefebvre, editors. Elsevier, Amsterdam. 31–42.

27. Reunanen, A., H.K. Åkerblom, and J. Tuomilehto. 1988. High incidence of insulin-dependent diabetes mellitus (IDDM) in children in Finland. *Arctic. Med. Res.* 47(Suppl. 1):535–539.

28. Dahlquist, G., and L. Mustonen. 1994. Childhood onset diabetes: time trends and climatological factors. *Int. J. Epidemiol.* 23:1234–1241.

29. Svejgaard, A., B.K. Jakobsen, P. Platz, L.P. Ryder, and J. Nerup. 1986. HLA associations in insulin-dependent diabetes: search for heterogeneity in different groups of patients from a homogenous population. *Tissue Antigens.* 28:237–244.

30. Caillat-Zucman, S., H.J. Garchon, J. Timsit, R. Assan, C. Boitard, I. Djilali-Saiah, P. Bougnères, and J.F. Bach. 1992. Age-dependent HLA heterogeneity of type 1 insulin-dependent diabetes mellitus. *J. Clin. Invest.* 90:2242–2250.

31. Schernthaner, G., J.E. Banatvala, W. Scherbaum, J. Bryant, M. Borkenstein, E. Schober, and W.R. Mayr. 1985. Coxsackie-B-virus-specific IgM responses, complement-fixing islet-cell antibodies, HLA DR antigens, and C-peptide secretion in insulin-dependent diabetes mellitus. *Lancet.* 2:630–632.

32. Eberhardt, M.S., D.K. Wagener, T.J. Orchard, R.E. LaPorte, D.E. Cavender, B.S. Rabin, R.W. Atchison, L.H. Kuller, A.L. Drash, and D.J. Becker. 1985. HLA heterogeneity of insulin-dependent diabetes mellitus at diagnosis. The Pittsburgh IDDM study. *Diabetes.* 34:1247–1252.

33. Landin-Olsson, M., J.P. Palmer, Å. Lernmark, L. Blom, G. Sundkvist, L. Nyström, and G. Dahlquist. 1992. Predictive value of islet cell and insulin autoantibodies for type 1 (insulin-dependent) diabetes mellitus in a population-based study of newly-diagnosed diabetic and matched control children. *Diabetologia.* 35:1068–1073.

34. Hagopian, W.A., C.B. Sanjeevi, I. Kockum, M. Landin-Olsson, A.E. Karlsen, G. Sundkvist, G. Dahlquist, J. Palmer, and Å. Lernmark. 1995. Glutamate decarboxylase, insulin, and islet cell antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. *J. Clin. Invest.* 95:1505–1511.