No Evidence for Genetically Determined Alteration in Insulin Secretion or Sensitivity Predisposing to Type 1 Diabetes

A study of identical twins

Mohammed I. Hawa, BSc
Riccardo Bonfanti, MD
Christina Valeri, MD

Michela Delli Castelli, MD
Huriya Beyan, PhD
R. David G. Leslie, MD

OBJECTIVE — To determine whether inherited changes in insulin secretion or sensitivity could predispose to type 1 diabetes, we studied identical twins of type 1 diabetic patients.

RESEARCH DESIGN AND METHODS — We studied prospectively a consecutive series of 27 identical twins of patients with type 1 diabetes who were initially nondiabetic, as well as 14 control subjects, over a period of 18 years. Of these 27 twins, 13 remain nondiabetic (now estimated at low disease risk) and 12 developed diabetes (pre-diabetic twins). Subjects were tested when not diabetic on at least two occasions with an intravenous glucose tolerance test (IVGTT), and we estimated insulin secretion as first-phase insulin response (FPIR), glucose clearance ($K_g$), and insulin sensitivity both by homeostasis model assessment of insulin resistance (HOMA-IR) and relative to insulin response by the basal HOMA-IR-to-FPIR ratio.

RESULTS — Twins now at low risk and control subjects had similar fasting blood glucose and insulin levels, FPIR, $K_g$, HOMA-IR, and HOMA-IR-to-FPIR ratio. In contrast, pre-diabetic twins compared with control twins had higher fasting insulin levels (10.3 ± 6.0 vs. 4.6 ± 4.0 mIU/ml), lower FPIR (245 ± 129 vs. 796 ± 622 mIU·min⁻¹·10 min⁻¹), lower $K_g$ (1.5 ± 0.6 vs. 2.6 ± 0.8% per min), and higher HOMA-IR-to-FPIR ratio (0.007 ± 0.005 vs. 0.001 ± 0.0009) (all $P < 0.01$).

CONCLUSIONS — These observations in low-risk nondiabetic identical twins failed to identify a familial alteration in either insulin secretion or sensitivity predisposing to type 1 diabetes.

Diabetes Care 28:1415–1418, 2005

Type 1 (insulin-dependent) diabetes is due to the destruction of the insulin-secreting cells of the islets of Langerhans and is probably caused by environmental factors operating in a genetically susceptible host to initiate a destructive immune process (1). At diagnosis of classic type 1 diabetes, the islets are heavily infiltrated with lymphocytes and insulin secretion is absent or markedly reduced (2). The disease process tends to be slow and progressive such that patients have immune changes detectable many months, even years, before the clinical onset of type 1 diabetes.

The most widely held view is that environmental factors induce this destructive immune response, which targets the insulin-secreting cells. An alternative view is that initially either the altered insulin secretion or insulin sensitivity is genetically determined, thereby predisposing to the destructive process. In support of this latter hypothesis, type 1 diabetes is associated with a polymorphism of the promoter region of the insulin gene and both decreased insulin secretion and decreased insulin sensitivity can be detected in the pre-diabetic period (3,4). Furthermore, changes in growth and weight are associated with risk of progression to diabetes, an observation that led to the accelerator hypothesis (5).

To test the proposal that genetically determined changes in insulin secretion or sensitivity predispose to type 1 diabetes, we studied prospectively the identical twins of patients with the disease. A cohort of such identical twins was studied over a minimum period of 18 years, by which stage we could identify those twins who developed diabetes and, with a high degree of certainty, those who are now unlikely to do so. Identical twins can differ in their genes, but these differences are not genetically determined through germ-line genes, as they result from factors such as somatic mutations and gene methylation. Thus, similarities between identical twins could be due to shared genetic or nongenetic factors, but differences must be nongenetically determined. Therefore, if a metabolic change in pre-diabetic twins is genetically determined, we would predict that identical twins of patients with type 1 diabetes,
who remain nondiabetic, should also show the metabolic change.

**RESEARCH DESIGN AND METHODS** — The British Diabetic Twin Study includes data on identical twins. To date we have 368 pairs, of whom one or both have diabetes. Twins were ascertained prospectively between 1982 and 1989 in a consecutive series because they were diabetic and not because they were twins and because 1) the index twin had type 1 diabetes according to standard criteria (6–8), 2) the co-twin was not diabetic and initially tested within 6 years of the diagnosis of the index twin, 3) at least two intravenous glucose tolerance tests (IVGTTs) were subsequently performed on each co-twin, and 4) follow-up in twins remaining nondiabetic was for at least 18 years after diagnosis of the index twin.

Twins were followed prospectively until either the end of the study (July 2004) or development of diabetes. The nondiabetic twins were tested at least annually when they were within 6 years of diagnosis of diabetes in their index twin, every 4 months if we thought they were at high risk of developing diabetes, and every 2–3 years thereafter. For this study in the series of 27 nondiabetic identical co-twins of type 1 diabetic patients, all but one twin were <35 years of age at the time of inclusion. Control subjects were obtained from the local population, had no family history of diabetes, were taking no drugs, had no clinical signs or symptoms of illness, were eating their normal diet at the time of testing, were not associated with the hospital, and were selected to achieve a similar distribution of age and BMI to the twins. All nondiabetic twins and control subjects initially had an oral glucose tolerance test (glucose was given as 75 g or 1.75 g/kg, whichever was less) to confirm that they were not diabetic according to the National Diabetes Database criteria and the American Diabetes Association criteria (6–8). All twins and control subjects were tested for autoantibodies to GAD and the insulinoma-associated protein 2 (IA-2) antigen as previously described (9). Monozygosity was established in all twin pairs as previously described (10).

Characteristics of the subjects tested are shown in Table 1. All 27 nondiabetic twins (mean age 21.1 ± 8.3 years) and 14 control subjects (mean age 21.1 ± 7.8 years) were followed prospectively. During follow-up of the 27 nondiabetic twins, 12 developed diabetes (mean age 17.3 ± 5.8 years at initial test and 22.3 ± 8.2 years at diagnosis) with a mean follow-up to diagnosis of 3 years. These twins are referred to throughout the text as pre-diabetic twins, and all but one of them developed type 1 diabetes without an identified period of noninsulin-requiring diabetes; one twin still does not require insulin 9 years after clinical diagnosis of diabetes but has GAD autoantibodies. We estimate here that all 15 twins who remain nondiabetic are at low disease risk (<1% risk) as they are >18 years (mean 26.0 ± 7.0 years) from the diagnosis of the index twin with normal glucose tolerance and without diabetes-associated autoantibodies to GAD and IA-2 (9,10).

At the end of the study period, we therefore had three groups: 12 pre-diabetic twins, 15 low-risk nondiabetic twins, and 14 normal healthy control volunteers. Twins at low risk and control subjects were tested initially and again 6 years later with an IVGTT. The mean follow-up for the control subjects was 6.0 ± 1.7 and for the twins was 6.0 ± 1.3 years. BMI at the second test was 22.8 ± 2.7 and 23.4 ± 3.4 kg/m², respectively. The difference in initial mean age between the pre-diabetic twins and low-risk twins was not significant. All the subjects gave informed consent, and the study was approved by the ethical committee at St. Bartholomew’s Hospital.

All subjects were studied in the supine position at least 15 min after a venous cannula was inserted into the antecubital vein under local anesthesia as previously described (11). Intravenous glucose, using 20% dextrose at 0.5 g/kg body weight, was infused into the vein as described. All blood samples were collected and stored at −20°C for the measurement of whole blood glucose and serum samples for the analysis of insulin and autoantibodies to GAD and IA-2. Insulin autoantibodies were not analyzed as they do not influence estimates of low disease risk in our twins (M.I.H., R.D.G.L., unpublished observations).

Whole blood glucose was measured by the glucose oxidase method (Yellow Springs Analyser, Yellow Springs, OH). Serum insulin levels were measured by a modification of the double-antibody radioimmunoassay method using monodinated tyrosine A14–labeled insulin (Amersham Pharmacia) (12). Blood glucose was measured immediately and samples for insulin were batched and measured at the same time. The mean within-batch coefficient of variation for our insulin assay was 4%.

Insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) based on fasting glucose and insulin values using mean levels from blood samples taken at least 10 min apart on a single occasion for each subject (13). We also estimated insulin sensitivity for the level of insulin secretion (HOMA-IR/first-phase insulin response [FPIR]), which is predictive of progression to type 1 diabetes (18). The intravenous glucose clearance rate (Kg) was used to estimate the glucose clearance rate using least squares, calculated as the slope of the regression line of the natural logarithm of glucose between 10 and 30 min after glucose infusion and expressed as percent per minute. FPIR was calculated after a glucose load (0.5 g/kg) as the area under the 0–10 min curve and above the fasting level.

**Statistical analysis**

Results are expressed as means ± SD unless otherwise stated. Variables were normally distributed using the normality test of Kolmogorov and Smirnov. Comparisons of variables were performed using

---

**Table 1** — Characteristics of control subjects, twins at low risk, and pre-diabetic twins

<table>
<thead>
<tr>
<th></th>
<th>Control twins</th>
<th>Low-risk twins</th>
<th>Pre-diabetic twins</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Age at first test (years)</td>
<td>21.1 ± 7.5</td>
<td>24.1 ± 9.3</td>
<td>17.4 ± 5.8</td>
</tr>
<tr>
<td>Male (n)</td>
<td>9</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.7 ± 2.5</td>
<td>22.2 ± 3.2</td>
<td>20.1 ± 2.5</td>
</tr>
<tr>
<td>Autoantibodies to GAD or IA-2</td>
<td>All negative</td>
<td>All negative</td>
<td>All positive</td>
</tr>
</tbody>
</table>

Data are means ± SD.
RESULTS — Table 1 shows the initial characteristics of the 27 identical twins, including 12 twins who were pre-diabetic (all autoantibody positive) and 15 twins who remain low-risk nondiabetic twins (all autoantibody negative), as well as 14 normal healthy control subjects. All subjects had IVGTT on at least two occasions and all twins remaining nondiabetic, as well as control subjects, had these tests at least 6 years apart. For all subjects we estimated FPIR, $K_g$, HOMA-IR, and HOMA-IR-to-FPIR ratio, of which FPIR and HOMA-IR-to-FPIR ratio are known to be abnormal in the pre-diabetic phase (1,14,18). No nondiabetic subject had a fasting glucose ≥7.0 mmol/l (126 mg/dl) or a diabetic oral glucose tolerance test during the period of study.

Table 2 shows the metabolic results for the initial and final IVGTTs in the three groups of subjects. Twins at low disease risk and control subjects had similar fasting blood glucose and insulin levels, FPIR, $K_g$, HOMA-IR, and HOMA-IR-to-FPIR ratio.

In contrast, pre-diabetic twins compared with control subjects initially had higher fasting insulin levels (10.3 ± 6.0 vs. 4.6 ± 4.0 mU/ml; $P < 0.01$), lower FPIR (245 ± 129 vs. 796 ± 622 mU · ml⁻¹ · 10 min⁻¹; $P < 0.001$), lower $K_g$ (1.5 ± 0.6 vs. 2.6 ± 0.8%·min; $P < 0.005$), and higher HOMA-IR-to-FPIR ratio (0.007 ± 0.005 vs. 0.001 ± 0.0009; $P < 0.01$). These differences persisted throughout the pre-diabetic period for all values except fasting insulin (Table 2). The initial and final tests for the metabolic parameters in the three groups did not differ significantly, except that the FPIR fell significantly in the pre-diabetic twins ($P = 0.03$), whereas the HOMA-IR-to-FPIR ratio increased significantly in the control subjects ($P = 0.0008$) (Table 2).

CONCLUSIONS — Our observations in identical twins of patients with type 1 diabetes are not consistent with the hypothesis that reduced insulin secretion reflecting β-cell dysfunction is a genetic defect predisposing subjects to type 1 diabetes. We also did not find evidence for genetically determined reductions in insulin sensitivity predisposing to the disease. We did, however, find both reduced insulin secretion and reduced insulin sensitivity relative to levels of insulin secretion in pre-diabetic twins who went on to develop diabetes. Because changes in identical twins detected in the pre-diabetic period are not found in low-risk identical twins of patients with type 1 diabetes, it is likely that these changes are not genetically determined.

Our results suggest that the disease process causing type 1 diabetes is unlikely to be due to an inherited defect in either insulin secretion or insulin sensitivity, but the results do not exclude other factors that could accelerate the disease process or promote metabolic decompensation. Indeed, metabolic decompensation could result either from increased linear growth, which has been linked to diabetes risk, or from increased childhood obesity, which has been correlated with age at presentation (15–17). In line with this proposal, a recent study using the same methods we employed confirmed that the normal relationship between insulin sensitivity relative to insulin secretion is disrupted in those diabetes-associated autoantibody positive siblings who develop diabetes most rapidly (18).

Given the lack of identity of all diabetes susceptibility genes, the study of identical twins of patients with type 1 diabetes (provided they are not pre-diabetic) is the most appropriate method to test the hypothesis that inherited metabolic changes predispose to type 1 diabetes. In addition and in contrast to siblings, the disease risk in identical twins declines with time so that we are able to ascribe low risks to selected twins based on our 40-year prospective study (10). We cannot exclude the possibility that more sensitive techniques to assess insulin secretory capacity or insulin sensitivity might detect subtle changes in those twins who have not developed diabetes, but with this proviso we were unable to support the proposal that
Genetic predisposition to diabetes

inherited changes in insulin secretion or sensitivity could account for the genetic susceptibility to type 1 diabetes. In contrast, subjects with diabetes-associated autoantibodies show a broad spectrum of clinical and metabolic phenotypes. For example, individuals genetically susceptible to type 1 diabetes with GAD autoantibodies can have normal fasting glucose but either normal, impaired, or diabetic glucose tolerance, or, in adults, as with one of our pre-diabetic twins, frank clinical noninsulin-requiring diabetes (latent autoimmune diabetes of adults) with diabetes-associated autoantibodies show a broad spectrum of clinical and metabolic phenotypes. For example, individuals genetically susceptible to type 1 diabetes with GAD autoantibodies can have normal fasting glucose but with either normal, impaired, or diabetic glucose tolerance, or, in adults, as with one of our pre-diabetic twins, frank clinical noninsulin-requiring diabetes (latent autoimmune diabetes of adults) with a high risk of progression to insulin dependence (19,20).

Acknowledgments — This study was supported by the British Diabetic Twin Research Trust, the Juvenile Diabetes Research Foundation International, Diabetes U.K., and the Joint Research Board at St. Bartholomew’s Hospital. We thank the twins, their families, research fellows, and research nurses for their assistance throughout this project.

References
2. Foulis AK, Stewart JA: The pancreas in recent-onset type 1 (insulin-dependent) diabetes mellitus: insulin content of islets, insulitis and associated changes in the exocrine acinar tissue. Diabetologia 26:456–461, 1984
5. Wilkin TJ: The accelerator hypothesis: weight gain as the missing link between type 1 and type II diabetes. Diabetologia 44:914–922, 2001