Male Sex Increases the Risk of Autoimmunity but not Type 1 Diabetes

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OBJECTIVE — The goal of this study was to explore the role of sex on the prevalence of autoantibodies, protective genetic subtypes, β-cell function, and the incidence of type 1 diabetes in a population of first- and second-degree relatives of patients with type 1 diabetes (probands). We examined both the effect of the sex of the individual screened as well as the effect of the sex of the individual's proband on diabetes risk variables tested.

RESEARCH DESIGN AND METHODS — The Diabetes Prevention Trial-Type 1 has screened 93,188 relatives of type 1 diabetic patients from February 1994 to January 2002. After observing that more men than women were islet cell autoantibody (ICA) positive for the group as a whole, we further explored the role of sex by detailed analysis of variables in this population.

RESULTS — Our data suggest only an influence of sex on the type 1 diabetes disease process. After adjustment for race, age, and relationship to proband, male sex was associated with the appearance of autoimmunity, i.e., the presence of ICA and having two or more antibodies. There was no effect of sex on the presence of other autoantibodies, insulin secretion, results of oral glucose tolerance test, or development of diabetes.

CONCLUSIONS — Our finding that male sex conveys an independent increased risk for development of ICA and multiple antibodies, while at the same time finding no difference with respect to the development of diabetes, suggests that male relatives with the known risk factor of ICA are less likely than comparable female relatives to progress to overt disease, that the pathogenesis of type 1 diabetes among men is slower compared with women, or that women develop diabetes manifesting different antibody responses.

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Type 1 diabetes is believed to be caused by autoimmune destruction of pancreatic β-cells beginning long before the development of clinical symptoms. The presence of autoantibodies, such as islet cell autoantibodies (ICAs) and insulin autoantibodies (IAAs), and impaired β-cell function during this preclinical period provides important information about the natural history of disease and identifies subjects at risk for development of overt disease for intervention trials. Recently, a post hoc analysis of ENDIT data reported that sex was a significant determinant of islet autoimmunity, as ICA alone or in conjunction with GAD and or insulinoma-associated protein 2 antibodies were more common in men than women (1).

Whereas the increased risk of autoimmunity among men suggests that they are also at increased risk for diabetes, a 1993 review of the epidemiological literature finds that the incidence is sex neutral (2). In contrast, the DiMe study of Finnish subjects aged 0–14 years (3), a prospective Swedish population study of type 1 diabetes among subjects aged 15–34 years (4), and several other more recent studies reported a male predominance in type 1 diabetes presenting after age 15 years of age (5,6). A report of the Diabetes Epidemiology Research International Group suggested that the male predominance of disease might be seen only in populations with high incidence of disease (7).

The Diabetes Prevention Trial-Type 1 (DPT-1) screened 93,188 relatives of type 1 diabetic patients from February 1994 to January 2002. After observing that more men than women were ICA positive for the group as a whole, we further explored the role of sex by detailed analysis of demographic, autoantibody, and β-cell function variables as well as diabetes risk in this population. In that other studies have also indicated a higher prevalence of diabetes among offspring of fathers as compared with offspring of mothers with diabetes, we further explored the role of sex by examining both the effect of the sex of the individual screened as well as the effect of the sex of the individual's proband on diabetes risk variables tested as part of DPT-1.

RESEARCH DESIGN AND METHODS

— DPT-1 screened first- and second-degree relatives of individuals with type 1 diabetes who were diagnosed before 40 years of age. Only individuals aged ≤40 years were eligible for screening for cytoplasmic ICA. If a relative was found to be ICA positive (≥10 JDF [Juvenile Diabetes Foundation] units), they were staged with HLA-DQ typing, an intravenous glucose tolerance test (IVGTT), determination of IAAs, and an oral glucose tolerance test (OGTT). Therefore, HLA and IAA test results were available for individuals who were found to be ICA positive. As part of a separate ancillary study, screening samples were also tested for the biochemical antibodies (GAD and ICA512). All antibody results

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Abbreviations: DPT-1, Diabetes Prevention Trial-Type 1; FPIR, first-phase insulin response; IAA, insulin autoantibody; ICA, islet cell autoantibody; IDS, Immunology of Diabetes Society; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

reported herein are from the initial screening sample.

ICA assay

Cytoplasmic ICAs were determined on frozen sections of human pancreas by the DPT-1 ICA Core Laboratory (University of Florida, Gainesville, FL: February 1994 to September 1997 and October 1999 to present; Research Institute for Children, New Orleans, LA: September 1997 to October 1999) (8). Results of ≥10 JDF units were considered positive. In the Immunology of Diabetes Society (IDS) Combinatorial Autoantibody Workshop, reported in Orvieto in 1995, this ICA assay had a specificity of 100% with a sensitivity of 74.4% for new-onset diabetes patients younger than 30 years.

GAA and ICA512AA assay

GAA and ICA512 autoantibodies were measured simultaneously by combined GAA and ICA512 radioassay as previously described in the DPT-1 GAA and ICA512AA Core Laboratory at the Barbara Davis Center (9). The assay was performed in the 96-well filtration plates with autoantibody-bound ^{3}H-GAD65 and ^{35}S-ICA512AA precipitated with protein A sepharose. The cut points were set at indexes of 0.032 (mean ± 2 SD, GAA) and 0.049 (mean ± 6 SD, ICA512AA), the 99th percentile of 198 normal control subjects. The interassay coefficient of variation of the assays is 6.5 and 9.6%, respectively, for GAA and ICA512AA assays. In the IDS Combinatorial Workshop for patients younger than 30 years, assay specificity was 99% for GAA and 100% for ICA512AA and sensitivity was 83.7% for GAA and 74.4% for ICA512AA.

IAA assay

IAAs were determined with a fluid-phase radioassay using polyethylene glycol precipitation in the DPT-1 IAA Core Laboratory at the Joslin Diabetes Center (10–13). The cut point was 39 nU/ml (mean ± 2 SD), which represented the 99th percentile of 151 normal control subjects. The interassay coefficient of variation is 10.3% at low positive values. In the IDS combinatorial workshop, the assay had a specificity of 91% and sensitivity of 49%.

β-Cell Function

An IVGTT was performed according to the ICARUS protocol (14,15). Fasting samples were drawn at −10 and −4 min through an intravenous line. A solution of 25% glucose (0.5 g/kg, maximum 35 g) was then administered intravenously over 3 min. Samples were collected at 1, 3, 5, 7, and 10 min after the end of the glucose infusion. Insulin and glucose levels were measured in the DPT-1 Beta Cell Function Core Laboratory (Seattle, WA).

First-phase insulin response (FPIR) was calculated as the sum of the IVGTT insulin values at 1 and 3 min. The 10th percentile of normal control subjects for siblings and offspring older than 8 years was 600 pmol/l. The 10th percentile for siblings and offspring younger than 8 years and the first percentile for parents was 360 pmol/l. FPIRs less than these values were defined as "low" and were used as the thresholds for eligibility for the parenteral insulin intervention trial.

An OGTT was performed after an overnight fast and insertion of an antecubital intravenous line, and samples were collected at −10 and 0 min. An oral glucose load was then administered (Fisherbrand Sun-dex; 1.75 g/kg, maximum 75 g). Blood samples were collected at 30, 60, 90, and 120 min after glucose consumption. C-peptide and glucose levels were measured in the DPT-1 Beta Cell Function Core Laboratory (Seattle, WA).

HLA typing

HLA typing was performed as part of the DPT-1 protocol at the first staging visit for ICA-positive relatives. HLA-DQA1 and DQB1 alleles were typed using PCR and sequence-specific oligonucleotide probes (16,17). HLA DQA1*0102, DQB1*0602 positive and negative control samples were included in all assays.

Development of diabetes

The diagnosis of diabetes was recorded if *1*) the subject reported having received a diagnosis of diabetes and had started insulin therapy, *2*) the fasting glucose level on an IVGTT or OGTT was >126 mg/dl, or *3*) the 2-h glucose level on an OGTT was >200 mg/dl on two separate occasions (18). Because we have previously reported no statistically significant difference in the time until diabetes among those with low FPIR to glucose stimulation randomized to observation versus parenteral insulin, we included all of these subjects in our analysis. However, investigation of subjects with ICA and IAA randomized to oral insulin versus placebo was an ongoing trial at the time of this analysis, and for purposes of this analysis, subjects were not included in this evaluation.

Statistical analysis

Because of the large numbers accessioned to the DPT-1 and the propensity to discover serendipitous relationships, the screened population was randomly divided into two groups in which relationships discovered in the first group (the test group) were then confirmed in the second group (the confirmation group). The χ^2 test was used to compare prevalence rates between sexes. Multivariate analyses were used to adjust for possible confounders (e.g., age, relationship to proband). Either because of the design of the DPT-1 screening protocol or the availability of serum, not all tests were evaluated on all subjects. Appropriate denominators are provided where necessary.

RESULTS — As of 1 January 2002, a total of 93,188 relatives of individuals with type 1 diabetes (probands) were screened for participation in the DPT-1. More women than men were screened, and men tended to be ~4 years younger (mean age 21.5 years for women and 17.5 years for men in the test group, $P < 0.0001$) in both the test and confirmation groups. The screened population was 80% white; blacks and Hispanics comprised 2.8 and 10.6%, respectively. There was a higher percentage of women than men among blacks (3.3 vs. 2.4%, $P = 0.001$) and a higher percentage of men than women among Hispanics (11.2 vs. 8.8%, $P = 0.001$) in the test group. However, only the higher proportion of women as compared with men among the blacks was statistically significant in the confirmation group. The family relationship of screened subjects to the proband with diabetes differed significantly between men and women in both the test and confirmation groups. Women tended to be more often the parent of a diabetic proband, whereas men were more likely to be the sibling, offspring, or a second-degree relative. These demographic characteristics were consistent in both the test and the confirmation groups (Table 1).

Autoantibodies

More men than women were ICA (3.9 vs. 2.8%, $P < 0.0001$), GAD (4.2 vs. 3.7%,

Table 1—*DPT-1 population demographics: test group*

	Test group			Confirmation group		
	Men	Women	*P* value	Men	Women	*P* value
Screened (*n*)	20,501	26,091		20,520	26,069	
Age at screening (years)	17.5 ± 12.9	21.5 ± 13.5	<0.0001	17.5 ± 12.9	21.5 ± 13.4	<0.0001
Race						
White	16,315 (79.6)	20,599 (79.0)	0.096	16,291 (82.5)	20,533 (81.7)	0.099
Black	500 (2.4)	872 (3.3)	0.001	499 (1.1)	822 (3.3)	0.001
Hispanic	2,295 (11.2)	2,296 (8.8)	0.001	2,309 (11.7)	3,001 (11.9)	0.339
Other	632 (3.1)	749 (2.9)	0.180	651 (3.3)	770 (3.1)	0.167
Unknown	759	952		771	944	
Relation to proband						
Sibling	8,161 (39.8)	9,758 (37.4)	0.001	8,291 (40.4)	9,791 (37.6)	0.001
Parent	3,754 (18.3)	7,070 (27.1)	0.001	3,766 (18.4)	6,953 (26.7)	0.001
Offspring	5,188 (25.3)	5,814 (22.3)	0.001	4,954 (24.1)	5,820 (22.3)	0.001
Second-degree relative	3,311 (16.2)	3,309 (12.7)	0.001	3,387 (16.5)	3,367 (12.9)	0.001
Other/unknown	87	14		122	138	
Screening antibodies						
ICA^+ (%)	3.9	2.8	<0.0001	3.6	3.1	0.001
GAD^+ (%)	4.2	3.7	0.01	3.9	3.6	0.16
$ICA512^+$ (%)	2.0	1.6	0.002	1.8	1.5	0.005
IAA^+ (%)	20.4	16.7	0.01	18.7	16.9	0.70
Positive for two or more (%)	2.4	1.7	0.001	2.1	1.8	0.004

Data are mean ± SD or *n* (%) unless otherwise noted.

$P = 0.01$), or ICA512 (2.0 vs. 1.6%, $P = 0.002$) positive on their screening sample in the test group. A sex difference in the prevalence autoantibodies was also found in the confirmation group for ICA and ICA512 ($P = 0.001$ and 0.005, respectively) but not for GAD ($P = 0.16$). IAAs were present in 20.4% of men and 16.7% of women ($P = 0.01$) in the test group, but this sex difference was not statistically significant ($P = 0.70$) in the confirmation group. Also, more men than women were positive for two or more antibodies (2.4 vs. 1.7%, $P = 0.001$) in the test group and in the confirmation group (2.1 vs. 1.8%, $P = 0.004$). The prevalence difference among the sexes with respect to ICA, ICA512, and the presence of two or more antibodies remained statistically significant ($P = 0.001$) in both the test and comparison groups when the comparison was made between those who were ICA-positive on at least two occasions.

In general, the prevalence of antibodies was greater among younger subjects (all $P < 0.005$), with the exception of IAA, which was not different when comparing subjects ≤14 vs. ≥15 years of age. In the test group, the ICA-positive rates for male and female subjects were 3.8 and 3.6%, respectively, for children aged ≤14 years ($P = 0.34$) and 3.3 and 2.7% for adolescents and adults aged ≥15 years ($P = 0.005$). The sex difference in both age groups was statistically significant in the confirmation groups in which the ICA-positive rates for men and women were 4.4 and 3.2%, respectively, for children aged ≤14 years ($P < 0.0001$) and 3.1 and 2.4% for adolescents and adults aged ≥15 years ($P = 0.0005$).

In a multivariate analysis, the greater proportion of ICA-positive and two or more antibody–positive men compared with women remained significant ($P < 0.0002$ in the test group and $P = 0.04$ in the confirmation group) after adjusting for race, age, and relationship to the diabetic proband. The resulting adjusted odds ratio for men as compared with women for ICA positivity was 1.38 (95% CI 1.25–1.54). However, the statistically significant higher prevalence of ICA512 among men noted in the univariate analyses in both the test and confirmation groups was no longer significant ($P = 0.09$) in the multivariate analysis in either group after adjusting for age.

ICA-positive subjects

According to protocol, ICA-positive subjects at screening were subsequently HLA screened. There were no significant sex differences in the prevalence of the "protective" HLA DQB1*0602 haplotype (7.8% men versus 9.8% women, $P = 0.21$) among those who were ICA positive at screening in the test group or in the confirmation group (6.7% men versus 9.5% women, $P = 0.69$) (Table 2). The results of IVGTTs were also not different between the sexes; 28.1% of women and 27.8% of men had FPIRs below the age-adjusted threshold ($P = 0.86$ in the test group and $P = 0.52$ in the confirmation group), and 1.9% of women and 1.3% of men had fasting glucose levels higher than the American Diabetes Association criterion for diabetes ($P = 0.37$ in the test group and $P = 0.43$ in the confirmation group). Excluding those who had the HLA DQB1*0602 haplotype, there were no sex differences in the results of those who underwent an OGTT; 72.0% of women and 74.9% of men had normal glucose tolerance, 15.9% of women and 14.6% of men had impaired fasting glucose or impaired glucose intolerance, and 12.2% of women and 10.5% of men had glucose values higher than the American Diabetes Association criterion for diabetes in the test group. The results were nearly identical in the confirmation group.

Table 2—*ICA-positive subjects*

	Test group			Confirmation group		
	Men	Women	*P* value	Men	Women	*P* value
Screened (*n*)	20,501	26,091		20,520	26,069	
DQB0602	53/676 (7.8)	60/611 (9.8)	0.21	58/653 (6.7)	63/662 (9.5)	0.69
Low FPIR	178/641 (27.8)	163/581 (28.1)	0.91	165/626 (26.4)	172/624 (27.6)	0.63
OGTT (*n*)	267	189		242	216	
Normal	200 (74.9)	136 (72.0)	0.48	180 (74.4)	156 (72.2)	0.60
Impaired fasting glucose	3 (1.1)	0		2 (0.83)	4 (1.9)	
Impaired glucose tolerance	36 (13.5)	30 (15.9)	0.48	39 (16.1)	35 (16.2)	0.98
Type 1 diabetes	28 (10.5)	23 (12.2)	0.57	21 (8.7)	21 (9.7)	0.70

Data are *n* (%) unless otherwise noted.

Multiple antibody–positive subjects

A total of 919 subjects in the test group had two or more antibodies at screening, and 903 subjects in the confirmation group had two or more antibodies. Multiple antibody–positive subjects tended to be younger than the population screened overall and for both men and women separately. As in the overall population, men positive for two or more antibodies tended to be younger than women (mean age 14.3 vs. 17.4 years, $P = 0.0002$). This sex difference was found in the confirmation group as well ($P = 0.0025$). A higher proportion of women (17.8%) than men (10.1%) were the parents of the diabetic proband in the test set, but unlike the whole population, a sex difference in the confirmation set was not found (12.1 vs. 14.5%, $P = 0.08$). Sex differences in the prevalence of ICA (83.4% of men versus 74.3% of women) and GAD (86.9% of men versus 90.9% of women) were statistically significant at $P = 0.001$ and $P = 0.05$, respectively, in the test set but were not significant in the confirmation data set ($P = 0.36$ and $P = 0.49$, respectively). There were no statistically significant sex differences in the prevalence of ICA512 in either dataset among those with two or more antibodies.

Whereas significantly more men than women were ICA positive, women tended to have higher median JDF titers (160 vs. 80 JDF units) in the test dataset at $P = 0.002$, but the difference was not statistically significant in the confirmation dataset ($P = 0.83$). On the other hand, the mean GAD titer for women was consistently higher ($P = 0.0001$) than that for men in both the test and confirmation datasets (0.59 vs. 0.47 and 0.61 vs. 0.50, respectively).

Time until diabetes

The increased prevalence of antibodies among male relatives of probands with type 1 diabetes would also suggest increased risk of diabetes. There was a statistically significant increase in the risk of diabetes in men in both the test and the confirmation groups. However, this difference was not statistically significant ($P = 0.42$ and $P = 0.58$) when the analysis was adjusted for age at screening and the occurrence of ICA and ICA512.

Offspring of diabetic probands

The prevalence of antibodies was statistically significantly higher ($P = 0.001$, $P = 0.01$, and $P = 0.002$, respectively) among children of fathers with diabetes ($n = 5,673$) as compared with the prevalence of these antibodies among children of mothers with diabetes ($n = 5,260$) in the test data group (ICA: 3.6 vs. 2.34%, GAD: 4.1 vs. 3.1%, and ICA512: 1.6 vs. 1.0%). However, when analyzed in the confirmation data group, the difference in the prevalence was in the same direction but no longer significant ($P = 0.1$, $P = 0.14$, and $P = 0.16$, respectively). There was no statistical significance in the prevalence of ICA comparing the male and female offspring of mothers with diabetes ($P = 0.3$) or fathers with diabetes ($P = 0.10$) in the test group and in the confirmation group ($P = 0.4$ and $P = 0.8$, respectively). This was true regardless of whether the parent was diagnosed with diabetes before adulthood.

CONCLUSIONS — Our data suggest only a subtle influence of sex on the type 1 diabetes disease process. With respect to the effect of the sex of the subject, univariate analysis demonstrated that men were more frequently ICA positive and ICA512 positive at screening. There was no effect of the sex on the presence of other autoantibodies, insulin secretion, results of OGTTs, or development of diabetes.

The most consistently reported sex effect in type 1 diabetes is that offspring of men with diabetes are more likely to have diabetes-related autoantibodies (19) and higher risk for disease (20–22) than offspring of women with diabetes. These studies were performed among populations of 240–2,000 offspring. In contrast, using data from 21,579 offspring, we did not demonstrate any consistent (i.e., in both the test and confirmation groups) effect of the sex of the parent on ICA. A report from Austria also demonstrated no sex differences in risk for diabetes among families with only one child with the disease (23).

Our method of analysis demonstrates important caveats in post hoc analysis of large sample sets. Understanding the effect of sex on the diabetes disease process was not designed as an aim of the DPT-1. It was upon the observation that there was a preponderance of male subjects in the staging and intervention parts of the program that we decided to explore the issue further. We used the "split-halves" approach to our dataset, testing the two hypotheses that the sex of the subject was an independent risk factor in the diabetes disease process and that the sex of the proband either of the group as a whole or among parents only was an independent risk factor. We then examined the same hypotheses in the second, or confirmation, group. Such an approach led to some differences in our conclusions than would have otherwise been the case. For

example, in the test group, more offspring of fathers with diabetes were antibody positive, as compared with offspring of mothers with diabetes, a finding with *P* values of 0.1–0.001. However, in the confirmation group, the proportion of offspring of fathers with diabetes that were antibody positive was not significantly higher than offspring of mothers with diabetes. Similarly, in the test group, we were particularly interested in the observation that children who were male offspring of fathers with diabetes were more often ICA positive than female offspring of fathers with diabetes (4.0% male offspring versus 3.2% female offspring, $P = 0.1$). This result was in contrast to a published report suggesting that the parent confers increased risk to the child of the opposite sex. In the confirmation group, there was no statistically significant difference between the sexes of offspring ($P = 0.8$), and the trend was the opposite direction than in the test group (i.e., female offspring of fathers with diabetes were more often ICA-positive than male offspring of fathers with diabetes [3.4% female offspring versus 3.3% male offspring]). On the other hand, this approach led to one of the same conclusions found in the post-hoc analysis performed by the ENDIT group, namely that men were more likely to be ICA positive than women.

Taken in the context of summary reports on the incidence of type 1 diabetes worldwide being gender neutral, our finding that there is a small independent risk in men for development of ICA and multiple antibodies presents an apparent paradox. It suggests that male relatives with autoimmunity are less likely then comparable female relatives to progress to overt disease. Plausible hypotheses to explain this observation might include that the pathogenesis of type 1 diabetes among men is slower than among women or that women develop diabetes manifesting different antibody responses. Although our data did not demonstrate a sex difference in the prevalence of GAD antibodies, GAD titers among those antibody-positive subjects were significantly higher among women. This finding is consistent with another report that GAD antibodies were less frequent and of lower titer in newly diagnosed male versus female subjects (24), which supports the latter hypothesis. In addition, our data found no sex differences in FPIR. Whereas a non-significant result must always be interpreted with respect to the numbers of subjects tested, there is not likely to be any clinical relevance to a finding not observed with more than 22,300 subjects in each of the test and confirmation groups followed for a median duration of 3.5 years (range 0–7 years).

Alternatively, recent information from Finland, Sweden, and other locations has reported a male predominance in disease incidence. This difference seems to increase with increasing age of the subject. In addition, data from the Pittsburgh Diabetes Epidemiology Research Group demonstrate not only an increasing incidence of disease over time but that this increase is greater among men (25). If an increasing incidence in men is occurring over time, it is then perhaps not unexpected that our study found an increase in ICA as a risk marker for disease and an increased incidence of diabetes in men that was accounted for by age and presence of ICA.

In conclusion, our analysis of the effect of sex of the subject or of the subject's proband on diabetes risk markers among the DPT-1 population group indicates that there is only a subtle effect of sex on the prevalence of autoantibodies but not on the incidence of diabetes.

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