Coexistent Autoimmunity in Familial Type 1 Diabetes: Increased Susceptibility in Sib-Pairs?

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Key Words

Type 1 diabetes · Familial type 1 diabetes · Coexistent autoimmunity

Abstract

Background: Type 1 diabetes (T1D) patients are at risk for additional autoimmune diseases (AID). **Objective:** To compare the characteristics of associated autoimmunity among familial (parent-offspring and sib-pair) subgroups and sporadic T1D patients. **Patients and Methods:** Data regarding AID in T1D patients and their nuclear family members were extracted from medical files of 121 multiplex T1D families (58 parent-offspring, 63 sib-pairs) and 226 sporadic controls followed between 1979 and 2008. **Results:** The prevalence of associated autoimmunity was similar in familial and sporadic cases (33.6 vs. 32.7%). The frequency of additional AID and percentage of patients with two or more coexistent AID were significantly higher among sib-pairs than parent-offspring ($p = 0.05$ and $p = 0.04$, respectively). The median time elapsed between diagnosis of T1D and occurrence of additional autoimmunity tended to be shorter in the sib-pairs. Only in familial cases did a positive autoimmune family background predict the development of coexistent autoimmunity (OR = 2.11, CI [1.0, 4.49] $p = 0.05$). **Conclusions:** Among sib-pairs with T1D, the higher prevalence of additional AID, the in-

creased number of diseases per person, and the relatively earlier appearance of associated AID suggest an increased susceptibility for coexistent autoimmunity in this subgroup. Positive family history for autoimmunity in multiplex T1D families increased their risk for co-occurrence of AID.

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Introduction

Type 1 diabetes (T1D), an immune-mediated disease developing in genetically susceptible individuals, is often associated with other autoimmune diseases (AID) [1]. According to reports in the literature, 15–30% of subjects with T1D have autoimmune thyroid disease (AIT) [1, 2], 4–9% have celiac disease (CD) [1, 3, 4], 0.3–5% have autoimmune gastritis [5], and 0.5% have Addison's disease [1, 6]. These AID may appear before, simultaneously with, or after the onset of T1D. Genetic and epidemiological data indicate that AID are also more frequently identified among family members of T1D patients as compared to the general population [7–9]. The main genetic determinants associated with T1D, AIT and CD map to the major histocompatibility complex, in particular DR3 and DQ2 [1, 10–13]. Recently, HLA class I and MICA alleles were found to be risk factors for the development of CD in T1D

[14]. Moreover, polymorphisms within genes outside the major histocompatibility complex, including PTPN22 and CTLA-4, have also been linked to T1D and AIT risk to varying degrees [1, 15–19]. On the basis of the hypothesis that the frequent coexistence of AID can be explained at least in part by a similar genetic background, we addressed the question as to whether additional AID are more prevalent in familial T1D patients. Reviewing the literature revealed a higher prevalence of T1D as well as other AID among offspring of T1D parents than in those of non-diabetic parents [20], and co-occurrence of autoimmunity in 20% of sibling pairs concordant for T1D [21]. To the best of our knowledge, as yet there have been no reports of studies analyzing associated AID in familial cases subdivided into parent-offspring and sib-pair subgroups.

In the past 30 years, 226 patients with familial T1D were followed in our tertiary center; these patients were categorized into parent-offspring (98 patients) and sib-pair (128 patients) subgroups. Recently, we published a retrospective-comparative analysis of the demographic and clinical characteristics at presentation of familial and sporadic T1D cases [22]. In the present study we extended our research to determine the co-occurrence of AID in familial T1D patients subdivided into parent-offspring and sib-pair subgroups as compared to that in sporadic T1D controls. Our primary objective was to assess the prevalence, type, sequence of occurrence and timing of appearance of associated autoimmunity. The secondary objective was to determine whether the frequency of AID among first-degree relatives differs in these subgroups.

Patients and Methods

Patients

Survey of the institutional registry of diabetic patients in our National Center for Childhood Diabetes yielded 226 familial T1D patients diagnosed and followed between 1979 and 2008. These patients belonged to 121 multiplex families: 58 parent-offspring families and 63 sib-pair families. All families met the following inclusion criteria: T1D in 2 or more first-degree relatives; T1D diagnosed after the age of 6 months. The control population was also extracted from the institutional registry, comprising 226 sporadic T1D patients who matched the familial cases by age, gender and year of diagnosis, and remained uniplex till December 2008. It should be noted that in accordance with the policy of our service, throughout most of the period covered by this study, young patients remained under follow-up until the age of 40 years and it was only in 2005 that this age limit was lowered to 30–35 years, so that actually all of the parents of the parent-offspring group were subject to the same screening procedures. Excluded from the study were patients with type 2 diabetes mellitus, genetic defects

of β -cell function (MODY syndromes, mitochondrial DNA mutations and Wolfram syndrome), drug- or chemical-induced diabetes, cystic-fibrosis-related diabetes, genetic syndromes associated with diabetes and insulin resistance/insulin deficiency (Prader-Willi syndrome, Down syndrome, Turner syndrome, Klinefelter syndrome).

Sixty-four patients of the sib-pairs also participated in the European Type 1 Diabetes Genetics Consortium (ET1DGC) study analyzing the genetic basis of T1D in affected sib-pairs families. Their DNA was analyzed for typing of HLA class II (DR, DQ, and DP) and CTLA-4.

The Familial T1D study protocol and the ET1DGC study protocol were approved by our Institutional Review Board.

Autoimmune Diseases

Due to the predisposition of T1D patients to develop other AID, the policy of our Diabetes Center during the last three decades had been to screen every T1D patient for AIT, CD and pernicious anemia (PA) at diagnosis and annually thereafter. These data were documented consistently in the medical files. Evidence for autoimmunity was defined serologically by the presence of positive organ-specific antibodies, with or without overt disease. The AID were classified as follows: thyroid autoimmunity-positive antithyroid antibodies with either normal or abnormal thyroid function; CD-positive serological tests confirmed by abnormal duodenal biopsy; latent CD-persistent positive serological tests with a normal duodenal biopsy; PA-positive parietal cell autoantibodies with low vitamin B₁₂ levels and anemia; 'other' AID-rheumatoid arthritis, systemic lupus erythematosus, chronic idiopathic thrombocytopenic purpura, psoriasis, vitiligo, inflammatory bowel disease, and autoimmune hepatitis.

Methods

Our institutional diabetes registry records every new-onset diabetic patient referred to our clinic. The registry is consecutive and includes demographic data (date of diagnosis, date of birth and gender). For each familial case, we randomly assigned the next age- and -gender-matched, consecutively diagnosed patient with sporadic T1D.

Extracted from the medical files of each patient were: date of birth, gender, number of nuclear family members, date of diagnosis of T1D; data on coexistent autoimmunity, including date of diagnosis and type of autoimmunity (thyroid, celiac, gastric, and 'others'); the presence of AID in first-degree relatives (type of disease and affected family member) reported at initial history-taking. Frequency of AID within each nuclear family was calculated by adding the total number of additional AID in the family divided by the number of first-degree relatives.

Laboratory Analysis

Antithyroid antibodies, antithyroid peroxidase and antithyroglobulin (normal reference <75 and <150 IU/ml, respectively) were initially measured by the hemagglutination method and in recent years by enzyme-linked immunosorbent assay (Orgentec Diagnostika GmbH, Mainz, Germany). Basal serum levels of TSH (0.4–4 mIU/l), free T₄ (FT₄) (11.8–24.6 pmol/l) and total T₃ (TT₃) (1.3–2.7 nmol/l); until 1994 TSH, FT₄ and TT₃ were measured using commercial RIA kits and thereafter by chemiluminescent enzyme immunoassay (Diagnostic Products Corp., Los Angeles, Calif., USA). Serological screening for CD in IgA-sufficient pa-

tients was previously performed using quantitative IgA-specific antibodies directed against anti gliadin. Serum IgA-anti gliadin antibodies were measured by ELISA (normal reference <80 U/ml); since 2003, antitissue transglutaminase is determined using enzyme immunoassay (normal reference <7.0 AU/ml) (Eu-tTG IgA umana; Eurospital SpA, Trieste, Italy). Positive samples were assessed for antiendomysial antibodies via a qualitative and semi-quantitative indirect immunofluorescence test (IMMCO Diagnostics, Inc., Buffalo, N.Y., USA). CD was confirmed by duodenal biopsy obtained in patients with positive serology and in highly suspicious IgA-deficient patients. Serum vitamin B₁₂ (193–982 pg/ml) was measured using commercial RIA kits until 1994 and thereafter by a commercial immunometric assay (Immulite 2000 Vitamin B₁₂; Diagnostic Products Corp.). Antiparietal cell antibodies (normal reference <10 U/ml) were measured by Varelisa enzyme immunoassay (Sweden Diagnostics GmbH, Freiburg, Germany).

Genotyping Methods

HLA analysis was performed in a central laboratory affiliated with the ETIDGC. The HLA genotyping was performed with a PCR-based sequence-specific oligonucleotide probe system as previously described [11]. A set of 24 SNPs selected in the CTLA4 gene region was genotyped by the Illumina GoldenGate technology; of these, 22 CTLA4 SNPs were genotyped by the Sequenom iPLEX technology as previously described [23].

Statistical Methods

All analyses were done using the SPSS version 16 program for Windows (SPSS, Inc., Chicago, Ill., USA) and the results are expressed as mean $\pm$ SD or median and interquartile range. Comparisons between unpaired groups (parent-offspring vs. sib-pairs) for variables with gaussian distribution were analyzed by the independent samples t test and for variables not having gaussian distribution by the Mann-Whitney U test. Comparisons between paired samples (familial subgroups vs. sporadic controls) were analyzed by the paired sample t test for variables with gaussian distribution and by the Wilcoxon signed-rank non-parametric test for variables not having gaussian distribution. Discrete variables were compared using Pearson's χ^2 . Kruskal-Wallis test was performed to compare time elapsed between diagnosis of T1D and various AID. Odds ratio (OR) was calculated for evaluating the association between the risk for coexistent AID and the following variables: gender, age at onset of diabetes, duration of diabetes, and presence of autoimmunity in the nuclear family. These calculations were done for the familial and sporadic groups as well as for each subgroup. Stepwise backward logistic regression analysis (Wald) was applied to determine variables significantly associated with prediction of autoimmune co-morbidities. A p value of <0.05 was considered significant.

Results

Additional AID in Multiplex T1D Families (fig. 1)

Additional autoimmunity was significantly higher among the sib-pairs than among the parent-offspring families (55.6 vs. 37.9%, respectively; $p = 0.05$). In 22.2%

Fig. 1. Additional AID in multiplex T1D families.

of the sib-pairs and in 8.6% of the parent-offspring families, coexistent AID was found in both the first-affected family member (in the parent-offspring family: the parent) and the second-affected family member ($p = 0.03$).

Associated Autoimmunity in Familial and Sporadic Patients (table 1)

Demographic Data. The mean duration of follow-up was comparable in the familial subgroups as well as in the sporadic group. Within the familial group, mean age at diagnosis was similar in the parent-offspring and the sib-pairs. Gender distribution revealed a male preponderance (60.2%) in the parent-offspring group and a slight female predominance (53.1%) in the sib-pairs ($p = 0.047$). These two parameters were not analyzed in the sporadic group as the patients were age- and gender-matched.

Additional AID. The overall frequency of additional AID as well as the percentage of patients with two or more coexistent autoimmunities were similar in the familial and sporadic patients. Among the familial T1D patients, the frequency of additional AID tended to be more prevalent in the sib-pairs than in the parent-offspring sub-

Table 1. Autoimmunity in T1D patients – comparison between familial and sporadic cases and between familial subgroups (parent-offspring and sib-pairs)

	Familial T1D	Sporadic T1D	p	Familial T1D P-O	Familial T1D sib-pairs	p
<i>Demographic characteristics</i>						
Number of patients	226	226		98	128	
Duration of follow-up, years	11.1 ± 8.7	11.3 ± 8.4	NS	11.5 ± 9.4	10.8 ± 8.3	NS
Age at diagnosis of T1D, years	10.8 ± 5.9	10.8 ± 5.5	NS	11.5 ± 6.9	10.2 ± 4.9	NS
Male:female, n (%)	119:107 (52.7:47.3)	119:107 (52.7:47.3)	NS	59:39 (60.2:39.8)	60:68 (46.9:53.1)	0.047
<i>Prevalence of additional AID</i>						
Affected patients, n (%)	76 (33.6)	74 (32.7)	NS	27 (27.6)	49 (38.3)	NS
One disease, n (%)	57 (25.2)	58 (25.7)	NS	23 (23.5)	34 (26.6)	NS
Two or more diseases, n (%)	19 (8.4)	16 (7.1)	NS	4 (4.1)	15 (11.7)	0.04
<i>Prevalence of the various types of AID</i>						
Thyroid autoimmunity, n (%)	47 (20.8)	38 (16.8)	NS	16 (16.3)	31 (24.2)	NS
Celiac disease, n (%)	14 (6.4)	17 (7.5)	NS	5 (5.1)	9 (7.0)	NS
Latent celiac, n (%)	12 (5.4)	13 (5.8)	NS	4 (4.1)	8 (6.2)	NS
Pernicious anemia, n (%)	16 (7.3)	17 (7.5)	NS	2 (2.0)	14 (10.9)	0.006
Others, n (%)	10 (4.5)	8 (3.5)	NS	4 (4.3)	6 (4.7)	NS
<i>Median time between diagnosis of T1D and various AID</i>						
Thyroid autoimmunity, years (IQR)	3.8 (0.0–11.2)	6.1 (1.2–18.5)	NS	6.4 (0.2–12.9)	2.9 (0.0–11.2)	NS
Celiac disease, years (IQR)	1.9 (0.0–10.7)	3.9 (1.0–6.7)	NS	2.5 (–1.2 to 14.0)	1.3 (0.0–8.1)	NS
Latent celiac, years (IQR)	4.8 (0.0–8.8)	3.4 (0.0–12.3)	NS	2.3 (0.0–12.7)	5.4 (0.0–8.8)	NS
Pernicious anemia, years (IQR)	12.5 (3.7–18.3)	7.3 (0.2–16.9)	NS	15.0 (13.9–16)	9.4 (3.5–19.9)	NS
Others, years (IQR)	2.8 (–5.2 to 12.1)	3.9 (–3.0 to 11.3)	NS	5.6 (–2.6 to 8.2)	–0.1 (–7.6 to 24.9)	NS

T1D = Type 1 diabetes mellitus; AID = autoimmune diseases; P-O = parent-offspring; NS = non-significant; IQR = interquartile range. Values are means ± SD.

group. The percentage of patients with two or more coexistent autoimmunities was significantly higher in the sib-pairs than in the parent-offspring group (11.7 vs. 4.1%, $p = 0.04$).

Type of AID. AIT was the most common co-morbidity in both familial subgroups and the sporadic group. The prevalence of AIT, CD, PA and ‘other’ coexistent autoimmunities was comparable in familial and sporadic groups. Within the familial group, each autoimmune co-morbidity tended to be higher in the sib-pair group; this difference reached statistical significance ($p = 0.006$) only for PA.

The most frequent combinations of additional AID were AIT with CD (10 patients, 5 familial and 5 sporadic) and AIT with PA (7 patients, 5 familial and 2 sporadic). Other combinations were: CD with PA, and CD or PA with ‘other’ autoimmunities.

Timing to Detection of AID Relative to T1D Diagnosis. Associated AID were detected before diagnosis, simultaneously with diagnosis, and after diagnosis of T1D in 8.1, 19.2, and 72.7%, respectively, of the familial cases and in 6.5, 17.2, and 76.3%, respectively, of the sporadic cases with a similar distribution in the familial subgroups. Al-

though statistically not significant, the median time elapsed between diagnosis of T1D and co-occurrence of other autoimmunity tended to be shorter in the familial than in the sporadic patients, particularly in the sib-pair subgroup. In both familial and sporadic groups, the sequence of occurrence of the various AID was similar, with CD appearing first, thyroid disease second and PA last. In the familial group, CD was detected significantly earlier and PA significantly later than the remaining AID ($p = 0.02$ and $p = 0.049$, respectively).

Autoimmunity in Nuclear Family Members (table 2)

The number of families positive for AID and frequency of diseases in each family were significantly higher in the familial group ($p < 0.001$ and $p = 0.001$, respectively). The prevalence and the frequency of AID per family tended to be higher in the sib-pair subgroup.

Positive autoimmunity in nuclear families was clustered into three categories: AIT, CD, and ‘other’ AID. While thyroid autoimmunity was the most common disease among both familial and sporadic nuclear families, thyroid and ‘other’ autoimmunities were significantly more prevalent in the nuclear families of the familial cas-

Table 2. Autoimmunity in nuclear family members – comparison between familial and sporadic cases and between familial subgroups (parent-offspring and sib-pairs)

	Familial T1D	Sporadic T1D	p	Familial T1D P-O	Familial T1D sib-pairs	p
<i>Nuclear family characteristics</i>						
Number of families	121	226		58	63	
Number of family members	6.3 ± 2.8	5.4 ± 2.1	0.001	5.1 ± 1.8	7.3 ± 3.0	<0.001
Families with AID, n (%)	57 (47.1)	43 (19)	<0.001	22 (37.9)	35 (55.6)	NS (0.08)
Frequency of AID, % (95% CI)	20.9 (16.4–25.4)	13.0 (10.5–15.5)	0.001	17.6 (11.8–23.4)	23.9 (17.1–30.7)	NS
<i>Prevalence of the various types of AID</i>						
Thyroid, n (%)	37 (30.6)	43 (19)	0.014	16 (27.6)	21 (33.3)	NS
Celiac, n (%)	19 (15.7)	27 (11.9)	NS	7 (12.1)	12 (19)	NS
Others, n (%)	30 (24.8)	16 (7.1)	<0.001	9 (15.5)	21 (33.3)	0.02

T1D = Type 1 diabetes mellitus; P-O = parent-offspring; AID = autoimmune diseases; NS = non-significant; CI = confidence interval. Values are means ± SD.

es ($p = 0.014$ and $p < 0.001$, respectively). The rate of two or three types of autoimmunity per family was significantly higher among familial than sporadic nuclear families (15.7 vs. 5.3%, $p < 0.001$).

Predictors of Autoimmune Co-Occurrence

Logistic regression analysis of additional AID clustered together showed that female gender was associated with coexistent AID in both familial and sporadic groups (OR = 2.28, CI [1.07, 4.85], $p = 0.03$ and OR = 2.26, CI [1.06, 4.85], $p = 0.04$, respectively). Presence of autoimmunity in the nuclear family was associated with coexistent AID only in the familial cases (OR = 2.11, CI [1.0, 4.49], $p = 0.05$). Age at diagnosis of T1D and duration of diabetes was not associated with co-occurrence of autoimmunity in either the familial or sporadic cases.

HLA Class II Typing

Among the 64 sib-pair T1D patients who underwent HLA typing, 28 patients had co-existent AID. The percentage of susceptibility alleles in the HLA class II DR3 region was more frequent among patients with associated AIT than those without AIT (82.4 vs. 56.5%, respectively; $p = 0.05$). The percentage of susceptibility alleles in the HLA class II DR3-DQ2 region was more frequent among patients with associated CD than those without CD (87.5 vs. 60%; the difference did not reach statistical significance, probably due to the small number of subjects tested). The frequency of DR3-DQ2 was higher in patients with a greater number of coexistent AID: with none, 57.1%, with one, 71.4%, and with two or more, 87.5%. The distribution of nucleotide polymorphisms in CTLA-4 was similar in patients with and without AIT.

Discussion

Despite the well-known association between T1D and additional autoimmunity, there have been relatively few reports on co-occurrence of AID within familial T1D categorized into parent-offspring and sib-pair subgroups. In this comparative analysis, we demonstrated that the frequency of additional AID and percentage of patients with two or more coexistent AID were significantly higher among the sib-pairs. Furthermore, the time elapsed from onset of T1D and detection of coexistent AID tended to be shorter in the familial cases, particularly those belonging to the sib-pair subgroup.

Our investigation of a large cohort of patients with familial T1D seen in one tertiary care center through the period of three decades has enabled us to delineate more clearly the characteristics of co-occurrence of autoimmunity in parent-offspring and sib-pair subgroups. In accordance with previous observations, associated autoimmunity was common among our T1D patients, with thyroid autoimmunity most frequently detected, followed by CD [1–5, 7, 8, 24]. Most associated AID appeared after diagnosis of T1D. In all subgroups, the sequence of appearance of the co-existent AID was similar, with CD appearing first, ATD second and PA last.

Although the pattern of co-occurring AID seemed to be similar in familial and sporadic T1D patients, within the familial group autoimmune co-morbidity was more prominent among the sib-pairs. The higher prevalence of additional autoimmunity among sib-pair families, the higher percentage of families in whom both T1D siblings were affected, the increased number of associated AID in the individual patient, and the relatively shorter time

elapsed from diagnosis of T1D to occurrence of AID suggest increased susceptibility in this subgroup. Genetic predisposition towards autoimmune conditions and environmental triggering factors have been suggested as the underlying cause for coexistent AID within families and individuals [25, 26]. The joint susceptibility for T1D and AIT and CD has been attributed to HLA class II genes, especially DR3-DQ2 alleles [1, 13, 27–29]. Indeed, HLA typing performed in 64 sib-pairs showed that HLA genes associated with AID were more frequently clustered among the sib-pairs. The relatively shorter median time to occurrence of additional autoimmune disease in the sib-pair subgroup is suggestive of the environmental effects. Shared exposure to novel environmental factors presumably instigated the development of coexistent AID in the genetically susceptible siblings.

Aggregation of AID among family members of T1D patients is a well-recognized phenomenon [1, 7–9]. The higher rate of autoimmune disease among first-degree relatives of familial T1D patients implies a genetic susceptibility in these families as well. Our data demonstrated a significantly higher prevalence of autoimmunity in first-degree relatives of familial T1D patients than in the sporadic patients. Furthermore, only in the familial group was high frequency of autoimmunity among nuclear family members found to be a predictor for development of additional AID. These findings emphasize the importance of the autoimmunity family history as a substantial predictor for co-occurrence of autoimmune disease in familial T1D patients.

It must be noted that our study had several limitations: the absence of laboratory evaluation confirming the re-

ported AID in nuclear family members, the lack of genetic analysis for the entire cohort, and the relatively small sample size. The trends indicated by some of the comparisons might have shown statistically significant differences in a larger cohort. Despite these limitations, the strength of this study stems from the fact that data were collected through a period of three decades in one institution, so that the entire cohort underwent similar clinical evaluation and laboratory screening.

To conclude, among multiplex T1D families, the higher prevalence of additional AID, the increased number of diseases per person, and the relatively earlier appearance of associated AID in the sib-pairs suggest an increased susceptibility for coexistent autoimmunity in this subgroup. Positive family history for autoimmunity in multiplex T1D families increased the likelihood of co-occurrence of AID.

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Disclosure Statement

None to declare.

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