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Predictors of associated autoimmune diseases (AAID) in families with type 1 diabetes (T1D). Results from the Type 1 Diabetes Genetics Consortium (T1DGC)

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Abstract

Background—Type 1 diabetes (T1D) is a clinically heterogeneous disease. The presence of associated autoimmune diseases (AAID) may represent a distinct form of autoimmune diabetes, with involvement of specific mechanisms. The aim of this study was to find predictors of AAID in the Type 1 Diabetes Genetics Consortium (T1DGC) data set.

Methods—3263 families with at least 2 siblings with T1D were included. Clinical information was obtained using questionnaires, anti-GAD and anti-IA-2 were measured and HLA-genotyping was performed. Siblings with T1D with and without AAID were compared and a multivariate regression analysis was performed to find predictors of AAID. T1D-associated HLA haplotypes were defined as the 4 most susceptible and protective, respectively.

Results—AAID was present in 14.4% of the T1D affected siblings. Age of diabetes onset, current age and time since diagnosis were higher, and there was a female predominance and more family history of AAID in the group with AAID, as well as more frequent anti-GAD and less frequent anti-IA2 positivity. Risk and protective HLA haplotype distributions were similar, though DRB1*0301-DQA1*0501-DQB1*0201 was more frequent in the group with AAID. In the multivariate analysis, female gender, age of onset, family history of AAID, time since diagnosis and anti-GAD positivity were significantly associated with AAID.

Conclusions—In patients with T1D, the presence of AAID is associated with female predominance, more frequent family history of AAID, later onset of T1D and more anti-GAD antibodies, despite longer duration of the disease. The predominance of certain HLA haplotypes suggests that specific mechanisms of disease may be involved.

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Keywords

immunoendocrinology; diabetes; thyroid autoimmunity; genomics

Introduction

Type 1A diabetes is a clinically heterogeneous autoimmune disease. Its course ranges from early, aggressive destruction of beta-cells to slow progression, where patients need insulin months to years after diagnosis. Although patients are typically young and lean, these features do not account for all, or even most affected subjects [1]. T1D is associated with an increased risk of associated autoimmune diseases (AAID), not only in the patients, but also in their relatives [2, 3]. Different AAID also cluster in individuals [4] and families; indeed, the risk of suffering an AAID is higher in 1st degree relatives of probands with T1D who already have an additional autoimmune disease [2]. Hence, the presence of AAID may represent a distinct form of autoimmune diabetes, with involvement of specific mechanisms.

The Type 1 Diabetes Genetics Consortium (T1DGC) is an international effort aimed at the study of the genetics and pathogenesis of T1D[5-7]. With thousands of families with T1D included from all over the world, this collection represents an extraordinary resource, not only of samples and genetic data, but also of associated clinical information. However, most of the reports published so far focus on the genetic results of the Consortium. In this study, we aim to identify both genetic and clinical predictors of the presence of AAID in subjects with type 1 diabetes. For this purpose, we selected two siblings per family, affected with type 1 diabetes, and compared those who did and those who did not have AAID. We performed multivariate analyses to identify factors associated with AAID in these subjects.

Methods

A total of 3304 families (441 trios) were included in the dataset analysed (available on 1st July 2009). Inclusion criteria have been described previously [5]. Briefly, an eligible family consisted of at least 2 siblings with T1D diagnosed before the age of 35 and treated with insulin within 6 months of diagnosis without an interruption longer than 6 months thereafter. Occasional exceptions were made to these criteria (through assessment by an eligibility committee) if other clinical data supported the diagnosis of type 1 diabetes. The parents of the affected sibpair, all affected and up to 2 unaffected siblings were invited to participate if available. In population groups with a low prevalence of type 1 diabetes, trio families, consisting of one affected patient and his/her parents, were also included. In order to avoid duplication, each family member was asked if they had participated in this study before a new inclusion was started. All of the participating centers were approved by the Office for Human Research Protection (Department of Health and Human Services, US). The local Ethics Committees approved the study and all participants signed a written informed consent before inclusion.

Clinical information was obtained using questionnaires delivered at each of the participating centers. Information was obtained directly from the participating family members and/or from their clinical records. Clinical data obtained included gender, ethnic background, age of onset, family history of diabetes, estimated body size at diagnosis (categorised as heavy, normal, thin) and self-reported AAID. AAID was considered to be present if any of the following was reported: thyroid, celiac or Addison's disease, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, vitiligo, psoriasis, inflammatory bowel disease, pernicious anemia or myasthenia gravis.

Glutamic acid decarboxylase (GAD65 [GADA]) and the intracellular portion of protein tyrosine phosphatase (IA-2_{ic} [IA-2A]) antibodies were measured in three central laboratories (Bingley et al, Bristol, UK (for the European and UK networks); Colman et al, Southbank, AUS (for the Asia-Pacific Network) and Eisenbarth et al, Aurora, CO, US (for the North American Network)) in serum from all the affected siblings. All laboratories used similar radio-binding assays, with different local standards, with values assigned by calibration against the World Health Organization (WHO) reference reagent. Results reported on samples included in the Diabetes Autoantibody Standardization Program (DASP) were compared, and standard methods for reporting in WHO units/mL were developed [8]. When antibody titres were above values in the standard curve, index estimations were chosen, since they gave a higher concordance among labs than extrapolation [8]. High antibody titres were defined as values above the respective standard curve.

HLA-genotyping was also performed centrally (Noble, Erlich et al, Oakland, CA, US, for the North American Network; Tait et al, Melbourne, Australia, for the Asia-Pacific Network and Carlson et al, Malmö, Sweden, for the European and UK Networks) in all participating family members, using a PCR-based, sequence-specific oligonucleotide probe system, as previously described [9]. Images of the results were scanned, and probe intensities were measured as pixel values and then imported into Sequence Compilation and Rearrangement Evaluation (SCORE) software for final genotype calling. T1D-related high-risk and protective haplotypes were defined according to previous data from the T1DGC [9], i.e. *DRB1*0405-DQA1*0301-DQB1*0302* (DR4), *DRB1*0401-DQA1*0301-DQB1*0302* (DR4), *DRB1*0301-DQA1*0501-DQB1*0201* (DR3) and *DRB1*0402-DQA1*0301-DQB1*0302* (DR4) as those associated with highest susceptibility and *DRB1*0701-DQA1*0201-DQB1*0303* (DR7), *DRB1*1401-DQA1*0101-DQB1*0503* (DR6), *DRB1*1501-DQA1*0102-DQB1*0602* (DR2) and *DRB1*1104-DQA1*0501-DQB1*0301* (DR11), as those associated with strongest protection.

Statistical analysis was performed using SPSS for Windows (SPSS Inc, Chicago, IL, US) and R. Continuous variables are described as median (range) and qualitative variables, as percentages. Differences between siblings with and without AAID were analysed using Wilcoxon-Mann-Whitney's test and chi-squared. Yates' correction was applied to the latter, except in 2x2 tables where the expected frequency for a cell was below 5, in which case Fisher's exact test was used. Differences in the distribution of high-risk, protective and single HLA-*DRB1-DQA1-DQB1* haplotypes were also analysed (Chi-squared with Yates' correction, or Fisher's exact test, as described above) in the subjects with unequivocal haplotypes.

To identify factors associated with AAID, a multivariate regression analysis (general additive model) was performed. In order to avoid interference by family size (i.e. bias in favour of factors present in larger families), only 2 affected siblings per family (the first 2 diagnosed, present in most families) were included in the analysis. Gender, age of onset, time since diagnosis, antibody positivity and presence of AAID in first degree participating relatives were included in the model as independent variables and analysed in all the families. In addition, the number of HLA haplotypes, associated with high risk of, or protection from T1D, were added to the model. Furthermore, the specific HLA haplotypes associated with higher risk of AAID in the descriptive analysis were included in a model together with the clinical predictors. For the latter analyses, we only included the families in whom *DRB1-DQA1-DQB1* haplotypes could be unequivocally inferred. In order to identify factors specifically associated with single disorders, the multivariate analysis was repeated using the most common AAID, i.e. thyroid and celiac disease, as dependent variables.

Results

Information about AAID status was available from 12973 of the 14620 participants. Unequivocal HLA haplotypes could be inferred in 11016 participants (5152 with type 1 diabetes), from 2711 families.

A total of 12.5% of the participants without and 14.7% of the participants with T1D had at least 1 AAID ($p=0.0002$). When relatives with and without T1D were compared, T1D tripled the risk [OR: 3.07 (2.00-4.72)] of AAID (Mantel-Haenszel chi-square, $p=2.03 \times 10^{-7}$), after adjusting for the presence of first-degree relatives with AAID.

Of the 1279 non-diabetic siblings analysed, 7.6% had AAID (see table 1). The latter were female (68.9% vs 50.5%, $p=0.001$) and had a first degree relative with AAID (60.3% vs 32.8%, $p<0.00001$), more frequently.

Information on AAID was available from 6262 of the 6270 siblings with T1D: 9.2% had thyroid disease, 2.7% celiac disease, 1.2% psoriasis, 1.1% vitiligo, 0.9% rheumatoid arthritis, 0.4% inflammatory bowel disease and 0.5% other disorders). Most ($N=807$; 12.9%) had only one AAID, 82 (1.3%) had 2, 8 (0.1%) had 3 and 2 (0.03%) had 4.

Differences between diabetic siblings with and without AAID

Age of onset of T1D, current age and time since diagnosis, were higher and there was a clear female predominance in the diabetic siblings with AAID (see table 1). GADA positivity was more frequent, whereas IA-2A positivity was less frequent in subjects with AAID and similar results were observed when antibody titres were considered instead of positivity/negativity (see table 1). Number of (diabetes-related) risk and protective *DRB1-DQA1-DQB1* haplotypes were similar in both groups: of the patients with AAID, 43.5% had one high-risk haplotype and 37.2% had two, as compared to 39.1% and 38.9%, respectively, in the group without AAID ($p=0.10$). None of the T1D siblings had 2 protective haplotypes and 1.8% of the siblings with and 1.4% of the patients without AAID had one ($p=0.57$).

When single HLA haplotypes were analysed separately, *DRB1*0301-DQA1*0501-DQB1*0201* (DR3), *DRB1*0401-DQA1*0301-DQB1*0301* and *DRB1*0404-DQA1*0301-DQB1*0302* (DR4) showed most significant susceptibility, whereas *DRB1*1502-DQA1*0102-DQB1*0502* and *DRB1*1502-DQA1*0101-DQB1*0501* were associated with highest protection against AAID (see table 3).

Factors associated with AAID

In the multiple regression analysis, female gender, age of onset, time since diagnosis, having a 1st degree relative with AAID and GADA positivity were significantly associated with AAID (see table 2). Similar results were obtained in the models using GADA and IA-2A titres instead of positivity (data not shown). In addition, similar results were obtained when all the affected siblings (and not only 2 per family) were included in the regression analysis and when only the first diagnosed or the second diagnosed siblings were analysed separately (data not shown). When the number of type 1 diabetes -associated, high-risk and protective HLA haplotypes were included in the model, none of them showed to be a significant predictor of AAID (data not shown). However, when the AAID-related risk haplotypes were included in the model, they, too, predicted the presence of AAID ($p=0.0018$, OR 1.25 (1.08-1.43) per haplotype), whereas the protective haplotypes did not, and GADA positivity lost its significance. Similar results were obtained when the most frequent AAID (thyroid and celiac disease) were analysed separately (instead of AAID globally) as dependent variables. For thyroid disease, the following were significantly associated with the disease: female gender ($p=2 \times 10^{-16}$), having a 1st degree relative with AAID ($p=2 \times 10^{-16}$), time

since diagnosis of type 1 diabetes ($p = 2 \times 10^{-16}$), older age of onset (4.45×10^{-6}), GADA positivity ($p < 0.005$) and number of AAID-associated risk HLA haplotypes ($p < 0.0005$) and, for celiac disease, female gender ($p = 2.26 \times 10^{-10}$), having a 1st degree relative with AAID ($p = 2.61 \times 10^{-10}$), time since diagnosis of type 1 diabetes ($p = 1.96 \times 10^{-7}$), younger age of onset ($p = 2 \times 10^{-16}$), IA-2A negativity ($p < 0.001$) and number of AAID-associated risk HLA haplotypes ($p = 4.37 \times 10^{-13}$).

Discussion

In the present study, we identified a distinct group of type 1 diabetes patients with AAID, who were predominantly female, had developed type 1 diabetes later and were more frequently GADA positive and had higher GADA titres, despite longer duration of the disease, than diabetic patients without AAID. The predominance of certain HLA haplotypes in this group suggests that specific mechanisms of disease might be involved.

The use of the T1DGC dataset to explore our hypothesis has two main advantages: the size of the sample and the homogeneity and high quality of data collection [10], including clinical information, but also GADA and IA-2A measurements, as well as a vast genetic database. Furthermore, the inclusion of parents in the study allows HLA haplotypes to be inferred to an extent, which is not possible in case-control studies. To date, most of the publications from the T1DGC have focused on the genetic risk of type 1 diabetes, but have not assessed the determinants of clinical presentation of the disease. In this report, we present a different approach to the analysis of the T1DGC data, focusing on the prediction of AAID in patients with type 1 diabetes. We are aware, however, that the study has some limitations: AAID were self-referred in most cases and no organ-specific auto-antibodies were measured (except for GADA and IA-2A). The prevalence of different AAID in this study is similar to that reported in other studies assessing clinically significant disease, but lower than in those measuring organ-specific antibodies [2, 11, 12]. An underestimation of the prevalence of AAID may have diluted the potential differences between the study groups. Therefore, the significant markers of AAID described in this study are probably true positive findings, although small but clinically significant predictors may have been lost to identification. Furthermore, the results may be applicable to the most prevalent AAID (i.e. celiac and thyroid disease), but not necessarily to other diseases which are less frequent in this population. In addition, the analysis was cross-sectional, performed in a very specific population of families including at least two siblings with type 1 diabetes, diagnosed before the age of 35 in most cases. Familial type 1 diabetes represents a minority of the patients suffering from the disease. Results are not necessarily expected to represent sporadic type 1 diabetes, or disease diagnosed later in life, where they would have to be confirmed. On the other hand, although most of the participants analysed are Caucasian [9], families have been included in many parts of the world, a fact, which would highlight factors common to very different environments.

Both female gender and age have previously been associated with autoimmune disease [12-15]. In addition, GADA, but not IA-2A, have been associated with other AAID, even in the absence of T1D [4, 16-18] and have been proposed as markers of more unspecific autoimmunity [19, 20]. GADA positivity and high GADA titres are associated with thyroid autoimmunity both in classical type 1 diabetes [4, 15] and in patients with LADA [21]. Unlike IA-2, GAD65 is found not only in pancreatic islets, but also in brain and, with lower expression, in thyroid and pituitary glands, kidney, liver, adrenal glands and gonads [22, 23]. GADA positivity is more frequent in carriers of DR3 (*DRB1**0301) and/or *DQB1**0201 (DQ2) alleles, whereas IA-2A positivity has been positively correlated with DQ8 (*DQB1**0302) and/or DR4 (*DRB1**0401, 0402, 0405) and negatively, with DR3 (*DRB1**0301)/*DQB1**0201 (DQ2) [20, 24, 25]. The DR4-DQ8 haplotype confers the highest

risk for type 1 diabetes [9] whereas DR3 confers a more broad-based risk for a spectrum of autoimmune diseases [26, 27]. In this setting, the results of the present study suggest that HLA genotypes may have an influence on the expression of IA-2A and GADA. Indeed, when AAID-associated HLA haplotypes were included in the multivariate analysis model, GADA positivity was no longer significantly associated with AAID, probably because HLA explained that association.

Some of the HLA haplotypes associated with AAID in the present study are also known to be associated with type 1 diabetes in the same direction (*DRB1*0301-DQAI*0501-DQBI*0201*, *DRB1*0404-DQAI*0301-DQBI*0302*, *DRB1*1502-DQAI*0101-DQBI*0501*, *DRB1*0403-DQAI*0301-DQBI*0302*, *DRB1*0101-DQAI*0101-DQBI*0501*), whereas others are inversely associated (*DRB1*0401-DQAI*0301-DQBI*0301*, *DRB1*0401-DQAI*0301-DQBI*0302*) or neutral (*DRB1*0404-DQAI*0301-DQBI*0402*, *DRB1*1502-DQAI*0102-DQBI*0502*) on type 1 diabetes risk [9]. When the most prevalent AAID, i.e. thyroid and celiac disease, were analysed separately as dependent variables, similar, though not identical, predictors were found. Indeed, age of onset of type 1 diabetes showed an inverse relationship (older for thyroid disease, younger for celiac disease). This suggests common mechanisms for multiple AAID, combined with disease-specific factors [28]. Some of these mechanisms are also common to type 1 diabetes, as shown by the increased risk of AAID conferred by the former in family members. However, the fact that multivariate analysis was performed in siblings with the disease should account for the effect of diabetes itself and allow us to identify factors, unrelated to diabetes, which increase the risk of other AAID.

Conclusions

In summary, the present investigation identifies a subgroup of patients with type 1 diabetes who have AAID, female predominance, later onset of the disease, high GADA positivity and an increased frequency of certain HLA haplotypes, including *DRB1*0301-DQAI*0501-DQBI*0201*. This may represent a specific form of the disease, maybe less aggressive on the beta-cell, but also less organ-specific, mediated by GADA.

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Table 1

Patient features in the whole dataset of siblings with type 1 diabetes and in those with and without associated autoimmune disease (AAID), as well as in those without diabetes

	Siblings without T1D	Siblings with type 1 diabetes		P**
		Total 6262	With AAID 899 (14.4%)	
Total	1279 (7.6% with AAID)	21.5 (1-76)	26.8 (3-76)	
Age (years)	22.6 (1-74)	49.6	68.1	<0.0005
Gender (% women)	51.8		46.5	<0.0005
Age of onset (years)	-	10.6(0-52)	11.9 (0-44)	<0.0005
Time since diagnosis (years)	-	10.9 (0-63)	14.9 (0-59)	<0.0005
AAID in participating 1 st degree relative (%)*	37.1	31.8	52.1	<0.0005
GADA positivity (%)	-	47.4	51.3	0.01
IA-2A positivity (%)	-	45.7	38.1	<0.0005
GADA titre (WHO U/ml)	-	9 (0-1426)	11 (0-1162)	<0.0005
IA-2A titre (WHO U/ml)	-	7 (0-2867)	4 (0-2381)	<0.0005
High GADA (%)#	-	15.3	21.5	<0.0005
High IA-2A (%)#	-	20.4	15.4	<0.0005

* Only one sibling (first diagnosed, if affected) per family included (N= 2193).

High antibody titres were defined as those above the standard curve for each antibody.

** For comparison between diabetic siblings with and without AAID

Table 2

Multiple regression analysis: predictors of AAID in siblings with type 1 diabetes

Variable	P	OR (95% CI)
Female gender	2×10^{-16}	2.36 (1.98-2.82)
Age of Onset (per year)	0.0014	Non-linear
Time since diagnosis (per year)	2.9×10^{-15}	Non-linear
GADA positivity	0.038	1.22 (1.02-1.46)
Family history of AAID (per participating relative)	2×10^{-16}	2.56 (2.13-3.08)
IA-2A positivity	0.075	0.85 (0.71-1.02)

Table 3

HLA haplotypes most significantly associated with risk of/ protection from associated autoimmune disease (AAID) in siblings with type 1 diabetes

HLA haplotype <i>DRB1-DQA1-DQB1</i>	Frequency of haplotype in siblings with AAID (%)	Frequency of haplotype in siblings without AAID (%)	OR (95% CI)	p
0301-0501-0201	51.80	47.53	1.19 (1.06-1.33)	0.0025
0401-0301-0301	6.39	4.73	1.37 (1.08-1.73)	0.0080
0404-0301-0302	11.49	9.54	1.23 (1.03-1.47)	0.021
0404-0301-0402	0.34	0.08	4.06 (1.04-14.11)	0.022
1502-0102-0502	0.13	0.77	0.18 (0.02-0.66)	0.0032
1502-0101-0501	0	0.40	0 (0-0.66)	0.0072
0403-0301-0302	0.61	1.27	0.48 (0.21-0.94)	0.027
0101-0101-0501	10.33	12.54	0.80 (0.67-0.96)	0.017
0401-0301-0302	31.95	34.57	0.89 (0.79-1.00)	0.051