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Familial clustering of autoimmune diseases in patients with type 1 diabetes mellitus

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Abstract

We investigated the familial aggregation of autoimmune diseases (AIDs) among first-degree relatives (FDR) of patients with type 1 diabetes mellitus (T1D). Relatives of 98 T1D patients defined according to the guidelines diagnosis of the American Diabetes Association and 113 matched controls without any AID, were interviewed using a questionnaire that sought information about demographic and medical characteristics including a list of 18 AIDs. Genetic analysis was performed using the program ASSOC and by calculating recurrent risk ratios. In cases, 25.5% of the families had at least one member having an AID, while in controls there were 9% (odds ratio [OR]: 3.96, 95% confidence interval [CI] = 1.74–9.0, $p = 0.0006$). An AID was registered in 8.3% of 312 FDR of patients as compared with 2.4% of 362 FDR in controls (OR: 3.56, 95% CI = 1.64–7.73, $p = 0.0008$). The most frequent AIDs registered in FDR of cases were autoimmune thyroid disease (AITD) and T1D, which disclosed coefficients of aggregation. These results indicate that AIDs cluster within families of T1D patients adding further evidence to consider that clinically different autoimmune phenotypes may share common susceptibility gene variants, which may act pleiotropically as risk factors for autoimmunity.

Keywords: Aggregation; Autoimmunity; Type 1 diabetes mellitus; Hypothyroidism; Genetics

1. Introduction

Autoimmune diseases (AIDs) are chronic conditions initiated by a loss of immunological tolerance to self-antigens. The chronic nature of many of these diseases results in a significant impact in terms of medical care utilization, direct and indirect economic costs, and quality of life. The estimated incidence of AIDs is about 90 per 100,000 person-years and their prevalence is about 3% of the population [1]. Almost all AIDs disproportionately affect middle-aged women, being one of the leading causes of death among this group of patients. The older the patient, the lower the male:female ratio becomes [1].

Although the etiology of AIDs is unknown, several factors are involved in the development of these diseases, including genetic and environmental factors [2,3]. Population studies have established that each population holds a mutational pool, in which most mutations individually (i.e. polymorphisms) will have mild effects; if not undetectable, but in

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combination with other alleles would favor or avoid autoimmune phenomena. Such interplay within genetic variants will generate a change in the measurable risk of developing an autoimmune phenotype. This characteristic is the main reason why AIDs are not inherited in a classical simple Mendelian way, but instead have a complex or yet unknown mode of inheritance [3]. Genetic contribution to AIDs is supported by the high rates of concordance, ranging from 15% to 60%, and by high recurrent risk ratios (λ_R) [4].

There is evidence indicating that some AIDs will concentrate within families. This includes not only cases of a single type of AID (several members having the same trait) appearing among siblings, twins and relatives of patients [5–8], but also several different ones (several family members with diverse AIDs) appearing [9–19], thus indicating that autoimmune phenotypes could represent pleiotropic outcomes of non-specific disease genes that underlie similar immunogenetic mechanisms. This common origin hypothesis for different AIDs is also supported by results of genome-wide scans showing that several loci may overlap in different AIDs [20,21], and by microarray expression profile studies disclosing a similar pattern of gene expression in different AIDs [22,23]. Finally, the multiple autoimmune syndrome, characterized by the presence of three or more AIDs in a single individual [24], is a clear example of how diverse phenotypes may be related to a single genotype.

Type 1 diabetes mellitus (T1D) is an organ-specific AID resulting from the damage of insulin-producing pancreatic β cells. This disease constitutes the earliest-onset AID, with a male:female ratio of 1:1 [25]. Similar to other AIDs, T1D is an immune-mediated disease that develops in genetically susceptible individuals, in whom one or more additional AIDs may coexist, autoimmune thyroid diseases (AITD) (i.e. Graves' disease or Hashimoto's thyroiditis) and celiac disease (CD) being the most prevalent [26–28]. First-degree relatives (FDR) are more predisposed to develop T1D and to have a higher proportion of autoantibodies compared to the general population [25]. Considering the interaction between AIDs that is summarized above and the fact that there is growing evidence supporting a common genetic origin for these diseases, the familial aggregation of autoimmunity within first degree relatives (FDR) of T1D patients was examined.

2. Patients and methods

2.1. Demographics of T1D patients and healthy individuals

Cases were children with T1D all of whom fulfilled the diagnostic classification criteria proposed by the American Diabetes Association (ADA) [29] and who belonged to our T1D cohort [30]. Their information on demographics and cumulative clinical manifestations over the course of disease were obtained by both chart review and discussion with the patient and was collected in a standard data collection form. A total of 98 patients with T1D were evaluated. Controls were 113 healthy children without any AID (see Appendix A) matched to cases by sex, age and ethnicity, and seen as outpatients by the same physicians.

The entire groups of individuals involved in this study were of Spanish ancestry and belonged to the population from the northwestern part of Colombia, South America (i.e. Paisa community). This population was established in the 16th–17th centuries and flourished in relative isolation until the late 19th century. The admixture between Paisa and African or Amerindian populations has been historically documented as low [31], with an ancestral ethnic component of 85% Caucasian and 15% Amerindian and in which the African contribution has been estimated as being not significant. Thus, historical and genetic evidence supports the usefulness of this population for genetic mapping [32].

2.2. Family collection

FDR of T1D patients and matched healthy individuals were interviewed following the methodology described by Priori et al. [10], using a standardized questionnaire that incorporated demographics and medical information including a checkpoint list of 18 AIDs (Appendix A). The diagnosis of AID was only considered reliable and consequently registered if made by a certified physician (i.e. endocrinologist, rheumatologist or an internist) and confirmed by chart review. FDR of T1D patients as well as FDR of the matched healthy individuals could not be considered as cases or controls. This research was accomplished in accordance with Resolution No. 008430 of 1993 from the Ministry of Health of the Republic of Colombia, was classified as research with minimal risk. The Ethics Committee of the CIB approved the present study.

2.3. Statistical and genetic analysis

Data were managed and stored using the SPSS program (v. 9.05 for Windows, Chicago, IL). Results are presented as means ± standard deviation (SD), and in percentages. Comparisons between means were performed by the Student's *t*-test, and those between percentages were performed by χ^2 test and two-sided Fisher's exact test as appropriate. Crude odds ratios (OR) were calculated with 95% confidence intervals (CI). A p value of less than 0.05 was considered as significant.

The ASSOC program in SAGE (Statistical Analysis in Genetic Epidemiology) was used to evaluate the existence of familial aggregation of AIDs, and to assess whether or not the presence of an AID affected proband correlates with the presence of a FDR who is affected by an AID, by simultaneously estimating familial variance components [33]. The model used incorporates familial correlations and arbitrary covariates assuming the correlation structure described by Elston et al. [34] and the regression model described in George and Elston [35]. For each individual (j), the model predicts parameters associated with a polygenic (G_j), family (F_j), marital (M_j), sibship (S_j) and a random environmental effect (E_j). The model is described as $h(y_j) = h(\beta^T x_j) + G_j + F_j' + M_j + S_j + E_j$, where h is a transformation of the dependent variable using the

standardized Box and Cox transformation [36] with the power parameter λ_1 and a shift parameter λ_2. Additionally, the polygenic effect and all random family and environmental effects are assumed to be normally distributed random effects with zero means. For each model, the data of one or more independent pedigrees was sampled at random to estimate the parameters of the model by maximum likelihood (ML) assuming a generalization of multivariate normality with or without the inclusion of a specified set of possible AIDs. The ML of the model is determined under two hypotheses: H_1 assumes the general model including all the covariates specified; while, H_0 excludes the test covariates. If L_1 and L_0 are the MLs under H_1 and H_0 respectively, then the likelihood ratio statistic is $2 \mid \ln(L_1) - \ln(L_0) \mid$. This joint test is asymptotically distributed as a chi-square with the number of degrees of freedom equal to the number of test covariates.

Furthermore, familial aggregation (λ_R) was calculated for first-degree relatedness (Parent/offspring and sibling/sibling pairs) using the formula $\lambda_R = K_{\text{Relative}}/K$, where K_{Relative} (K_R) was the prevalence for a specific degree of relatedness in the sample, and K was the prevalence in the control pedigree samples or the mean prevalence in the population [37]. In other words, two approaches where taken to examine λ_R. First of all, a relative-pair comparison between the prevalence of AIDs for the correspondent proband within each pedigree was calculated on the pedigrees for both T1D and healthy individual (case-control analysis). Secondly, previously reported prevalences of AIDs were considered [1,4,16,17,38–41]. These prevalences were used to obtain the λ_R values using the calculated prevalence for each specific degree-relative on the T1D affected proband pedigrees. Given the fact that prevalence information about AIDs in our population was not available, prevalences in the range of 0.1–0.5% were chosen [1,4,16,17,38–41]. Furthermore, 0.4% (4/1000) individuals for each AID and 2.5% (25/1000 individuals) for all AIDs taken together were selected as putative population prevalences [1,4,16,17,38–41]. Finally, since there was a subgroup of AIDs in the pedigrees of T1D patients that disclosed a low frequency, all AIDs were combined in order to determine the presence of familial aggregation for AIDs as a trait. These methods were extended to ascertain whether or not clustering of two or more autoimmune disorders in relatives of T1D patients increased the probability or risk for the presence of the disorder in the proband.

3. Results

3.1. Diverse autoimmune diseases in pedigrees of T1D patient and controls

In this study 98 T1D patients were examined. In the families cases, 25 (25.5%) presented at least one FDR having an AID compared with 9 (8%) in control families (OR: 3.96, 95% CI: 1.75–9; $p = 0.0006$) (Table 1). There were not differences within gender, with a female: male ratio of 1:1.

Specific AIDs in FDR of T1D patients and healthy individuals are shown in Table 2. There were a total of 26 (8.3%) AIDs among the 312 FDR in cases compared with 9 (2.4%) AIDs among 362 FDR in controls (OR: 3.56, 95% CI = 1.64–7.32, $p = 0.0008$), with a higher prevalence of AIDs in FDR females of T1D patients compared with FDR control females (OR: 4.52, 95% CI = 1.5–14.0, $p = 0.004$) (Table 2). Furthermore, T1D (OR: 20.24, 95% CI = 1.16–352.3, $p = 0.002$), autoimmune thyroid disease (AITD) (OR: 3.0, 95% CI = 1.15–7.82, $p = 0.02$) and all AIDs taken together as a trait (OR: 3.56, 95% CI = 1.64–7.32, $p = 0.0008$) were more prevalent in relatives of the cases than in relatives of controls (Table 2).

Table 1
Characteristics of patient's families with T1D and healthy individuals

Characteristic	T1D patients, $N = 98$ (%)	Controls, $N = 113$ (%)
Age (years)	8.8 ± 6.3	9.05 ± 4.22
No. of FDR	312	362
Families with ≤5 FDR	91 (92.3)	96 (84)
Families with 6–10 FDR	7 (7.1)	17 (15)
Families with a FDR having at least one AID		
Yes	25 (25.5)	9 (8)*
No	73 (74.4)	104 (92)

FDR, first-degree relatives; AID, autoimmune disease; T1D, type 1 diabetes mellitus; OR, odds ratio; CI: confidence interval.
*Comparison between cases and controls: OR: 3.96, 95% CI = 1.74–9, $p = 0.0006$.

The pedigree's general statistics for the 98 T1D patients and 113 healthy individuals are disclosed in Table 3. The mean pedigree size, standard deviation as well as the total number of relative pairs was obtained to calculate the prevalence for each AID. The analyses were restricted to FDR.

3.2. Familial AID correlation

By assessing if the presence of T1D in the proband correlated with the presence of an AID in a first-degree affected relative, the association for the occurrence of each individual AID was evaluated. Each model was weighted up every time a covariate (i.e. AID) was added to assess if the new trait would improve the likelihood of the primary phenotype by using the joint test, as described in the methods section. No significant correlation was observed for any of the examined AIDs, implying that none of the inspected models would explain the presence of the proband disease phenotype. Moreover, when all the AIDs were considered together, as a trait, no significant correlation was observed.

3.3. Familial aggregation (λ_R)

The prevalence for each AID as well as for all AIDs taken together for each pair of relatives (Parent/offspring, Sibling/sibling and total FDR) is disclosed in Table 4. Previously reported prevalences were also taken into account [1,4,16,17,38–41]. These calculated prevalences were used to obtain the familial aggregation for different degrees of relatives (Table 5). Additionally, using putative chosen

Table 2
Specific AIDs in first-degree relatives of T1D patients and healthy individuals

AIDs	AIDs in FDR of cases			AIDs in FDR of controls		
	All	F	M	All	F	M
Megaloblastic anemia	1	1	0	0	0	0
Systemic lupus erythematosus	1	0	1	0	0	0
Vitiligo	1	0	1	3	0	3
Type 1 diabetes	8	7	1	0*	0	0
Autoimmune thyroid disease	15	7	8	6**	4	2
No. of AID in FDR (%)	26/312*** (8.3%)	15/312 (4.8%)	11/312 (3.5%)	9/362 (2.5%)	4/362**** (1.1%)	5/362 (1.4%)

AIDs, autoimmune diseases; FDR, first-degree relative; T1D, type 1 diabetes mellitus; F, female; M, male.
*Comparing frequency of T1D in FDR among cases and controls: OR: 20.24, 95% CI = 1.16–352.3, $p = 0.002$.
**Comparing frequency of autoimmune thyroid diseases in FDR among cases and controls: OR: 3.0, 95% CI = 1.15–7.82, $p = 0.02$.
***Comparing frequency of AID in FDR of cases and controls (OR: 3.56, 95% CI = 1.64–7.73, $p = 0.0008$). 26 AID were observed in 25 FDR. There was one FDR having two AIDs. No FDR among controls had more than one AID.
****Comparing frequency of AID in females FDR of cases and controls: OR: 4.52, 95% CI = 1.5–14.0, $p = 0.004$.

prevalences (AID individually ($K = 0.4\%$) and all AIDs together (2.5%), λ_R were calculated (Table 5). Values supporting familial aggregation ($\lambda_R > 1.0$) were observed for both groups using both the data on pedigrees and putative chosen prevalences. Familial aggregation of AITD ($\lambda_R \sim 4.9 \pm 3.0$), T1D ($\lambda_R \sim 4.21 \pm 4.82$) and all AIDs taken together ($\lambda_R \sim 2.21 \pm 1.41$) was observed in T1D patients.

4. Discussion

These results indicate familial clustering of AIDs in patients with T1D in our population, and are consistent with previous results showing familial autoimmunity in other AIDs such as rheumatoid arthritis (RA) [9], systemic lupus erythematosus (SLE) [10,11], primary Sjögren's syndrome [12,13], polymyositis [14], juvenile RA [15], multiple sclerosis [16], vitiligo [17], and pemphigus [18].

Tait et al. [19] examined the presence of AIDs in British family members of patients with T1D. Similarly to ours, they observed that AITD was the most common AID among relatives. They also found that the prevalence of AIDs was higher in parents of T1D patients than in the general population, confirming the importance of family history as a significant risk factor for the development of T1D and supporting the hypothesis of shared etiological mechanisms for AIDs [19].

Table 3
Pedigree's general statistics for T1D patients and healthy individuals

Descriptors	Pedigrees	
	T1D	Healthy individuals
No. of pedigrees	98	113
Mean size ± SD	4.17 ± 1.35	4.33 ± 1.18
(Min, Max)	(3, 10)	(3, 8)
Pairs		
Parent/Offspring	426	518
Sibling/Sibling	211	246
Sister/Sister	56	13
Brother/Brother	41	34
Brother/Sister	114	41

T1D, type 1 diabetes mellitus.

AITD is the most consistently associated disorder with other AIDs including T1D [10,12,17,27,28]. Although CD may also be associated with T1D [26–28], this disease is extremely rare among our T1D patients [30]. However, most of the cases with CD may be subclinical or manifested at adulthood. Nevertheless, careful assessment of signs and symptoms suggesting CD was carried out (i.e. malnutrition, growth retardation, iron deficiency anemia). In addition, we have tested our cohort for immunoglobulin A anti-endomysium antibodies (by an indirect immunofluorescence method) and found only one positive case who was free of symptoms for CD (Anaya et al., unpublished results).

Autoimmune hypothyroidism was the most common disease encountered among FDR of our T1D patients as has been also reported in familial studies of Sjögren's syndrome [12,13], multiple sclerosis [16], vitiligo [17], juvenile RA [15], and SLE [10]. This finding supports previous analysis suggesting that AIDs (i.e. T1D and AITD) might be the consequence of pleiotropic effects of a single major gene on a polygenic background [2,9,12]. The lack of genetic information (i.e. genotypes) prevents us from drawing any conclusion about the specific role of loci in the susceptibility of AIDs. Nonetheless, the strong suggestions from previous studies allow us to point out the major histocompatibility complex (MHC), including both HLA and non-HLA [3,4], as one of the central loci contributing to T1D and AIDs. However, not all AIDs share the same genetic susceptibility or allelic spectrum. Thus, the genetic risk factors for AIDs may well consist of two forms: those that are common to many AIDs and those that are specific to a given disorder.

The next most commonly registered AIDs in FDR of T1D patients were T1D and SLE. Familial correlation was not obtained for these diseases because at least one of the maximizations was not available and thus no joint test could be performed. However, λ_R was observed for T1D but not for SLE (Table 5). CD was not observed in FDR of patients and controls. We cannot exclude an ascertainment bias since CD could be asymptomatic and test for specific antibodies or histopathological examination of the small intestinal endoscopic biopsy was not done.

Table 4
Prevalence of AIDs in T1D patient's and healthy individual's pedigrees

AIDs	K_{DM}[a]			K_{HI}			Reported K (population) [Ref.]
	P/O	SIB	REL	P/O	SIB	REL	
T1D	0.47	3.79	1.26	0.00	0.00	0.00	0,48 (UK, USA—Caucasian) [17] 0.19 (North Americans) [39] 0.34 (UK—Caucasian)) [41]
SLE	0.23	0.00	0.16	0.00	0.00	0.00	0.024 (USA—Caucasian) [1] 0.027 (UK—Caucasian) [16]
AITD	1.64	3.79	2.35	1.16	0.00	0.65	*Graves' disease* 0.65 (UK—Caucasian) [38,39] 0.80 (USA—Caucasian) [17,40] *Hashimoto thyroiditis* 0.80 (UK—Caucasian) [39] 1.15 (USA—Caucasian) [40]
VIT	0.00	0.00	0.00	0.58	0.00	0.33	0.40 (USA—Caucasian) [1,17]
All AIDs	2.11	8.06	4.08	1.74	0.00	0.98	2.5 (UK—Caucasian) [16]

AID, autoimmune disease; T1D, type 1 diabetes mellitus; AITD, autoimmune thyroid diseases; SLE, systemic lupus erythematosus; VIT, vitiligo.
[a] Data are given in percentages. Prevalences are disclosed between relative pairs ([P/O], Parent/offspring; [SIB], Sibling/Sibling; [REL] Relatives). K_{DM}: prevalence for AID in T1D patient pedigrees. K_{HI}: prevalence for AID in control pedigrees. K: prevalence in the general population.

Both T1D and AITD share similar susceptibility gene polymorphisms including HLA and non-HLA variants [4,20,42], which may account for the observed aggregation. Shared genetic factors are in fact the most likelihood cause for familial aggregation; however, it is important to keep in mind the fact that shared environmental factors can also explain this aggregation. For a specified relative type, a λ_R greater than one suggests familial aggregation of the disease, but does not identify whether genetic and/or environmental factors are aggregating [43]. Thus, a major strength of this study was the inclusion of healthy individuals matched by age, sex, origin and ethnicity, whose environmental conditions were similar to those of the patients.

The prevalence of AIDs among FDR of control individuals was 2.5% (Table 2), which is similar to the reported prevalence of such disorders in the general population [1] and in FDR of controls in other studies of familial autoimmunity [16,18]. Familial aggregation (λ_R) of AITD, T1D and all AIDs taken together was observed (Table 5). The λ_R obtained indicates how frequently an autoimmune trait is present in the sampled pedigrees depending on its distribution. The healthy individual's pedigrees ought to represent the general population given the conditions where each affected individual has been matched with an unaffected individual by age-, sex-, origin and ethnicity, who did not exhibit any history of AID.

Table 5
Familial aggregation (λ_R) of AIDs in T1D patients

AID	$\lambda_R = K_{DM}/K_{HI}$			$\lambda_R = K_{DM}/K$		
	$\lambda_{P/O}$	λ_{SIB}	λ_{REL}	$\lambda_{P/O}$	λ_{SIB}	λ_{REL}
T1D	NA	NA	NA	1.17	9.47	3.15
SLE	NA	NA	NA	0.57	0.00	0.40
AITD	1.41	NA	3.61	4.10	9.47	5.87
VIT	NA	NA	NA	0.00	0.00	0.00
AID[a]	1.21	NA	4.16	0.84	3.22	1.63

K_{DM}: prevalence for AID in T1D patient pedigrees; K_{HI}: prevalence for AID in healthy individual's pedigrees. K: chosen prevalence for the general population. Recurrent risk ratio ($\lambda_R = K_{DM}/[K_{HI}$ or $K]$), where R is the specific relative pair used (P/O, Parent/offspring; SIB, Sibling/Sibling; REL, Relatives); the ratio was calculated by a comparison between prevalence of T1D (K_{DM}) in patients depending on its first-degree relative disease or by using the chosen putative population prevalence for AID (data from Table 4), NA, data not applicable. Since no prevalences were observed for K_{HI}, a proper appreciation of λ_R could not be accomplished. Nevertheless, if taking these results into consideration the λ_R would disclose a high aggregation for those AIDs that were observed in patients but not in controls pedigrees.
[a] When taken together the chosen population prevalence (K) for AIDs was considered as 25/1000 individuals (2.5%) and for each individual AID 4/1000 (0.4%).

Whilst the different weighted models could have predicted how a trait would explain the presence of an AID in the proband when there is an FDR affected with an AID, the non-significant observed familial correlation among the examined models implies that the presence of each one of these autoimmune traits is not mathematically associated with the proband disease phenotype (i.e. AID). However, the lack of correlation with any of the AIDs might be explained by its familial distribution, frequency and late-age of onset. In fact, AIDs can exist subclinically for a significant period of time [6]. Since T1D is the earliest onset AID, prospective studies are necessary to accurately assess whether or not familial and shared autoimmunity arise during the patient or FDR lifetime.

We did not observe a predominant paternal inheritance of T1D within families as has been observed by others in T1D [19] as well as in other AIDs [10,12]. However, a significant predominance of familial autoimmunity among FDR females was registered. Consideration must be given to maternal transmission due to the high preponderance of AIDs in females compared with the general population [1].

In summary, our results indicate familial autoimmunity in T1D and sustain a common immunogenetic origin for diverse autoimmune phenotypes. As a corollary, results also emphasize the importance of the autoimmunity family history as a substantial risk factor for the development of T1D and other AIDs.

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Appendix A. List of AIDs investigated in the present study

No.	Autoimmune disease	Reference
1	Diabetes mellitus type 1	1
2	Systemic lupus erythematosus	2
3	Antiphospholipid syndrome	3
4	Rheumatoid arthritis	4
5	Sjögren's syndrome	5
6	Mixed connective tissue diseases	6
7	Ankylosing spondylitis	7
8	Scleroderma	8
9	Dermato-polymyositis	9,10
10	Crohn's disease or ulcerative colitis	11
11	Megaloblastic anemia	12
12	Hypothyroidism (Hashimoto)	13
13	Hyperthyroidism (Graves)	14
14	Psoriasis	15
15	Vitiligo	15
16	Primary biliary cirrhosis	16
17	Autoimmune hepatitis	17
18	Multiple sclerosis	18
19	Other	

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19. This was a free question asked by the physicians and included vasculitis and coeliac disease (i.e. malabsorption and the presence of anti-endomysium or anti-gliadin antibodies).

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