Molecular Epidemiology of Autoimmune Thyroid Disease

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Resumen

*Este documento presenta datos preliminares en relación a la prevalencia y factores de riesgo para la enfermedad tiroidea autoinmune en diabetes mellitus insulinodependiente encontrados en el registro 1950-1965 (n=669) de DMID del Hospital Infantil de Pittsburg. Los diabéticos vivos quienes participaron en la encuesta de seguimiento en 1990 (n=380) fueron reclutados para el estudio familiar de diabetes y enfermedades autoinmunes. Los padres y hermanos fueron también invitados para participar. A la fecha han sido evaluados 225 DMID y 597 padres y hermanos. El diagnóstico de enfermedad tiroidea autoinmune se basó en la evaluación clínica, la historia médica y las pruebas de laboratorio. La enfermedad grave era rara en esta cohorte (n=5). De cualquier forma, la tiroiditis de Hashimoto era común entre las mujeres. Las tasas de prevalencia oscilaban entre el 54% para DIMD y un promedio de edad <40 años al 75% en >50 años. De acuerdo con la estimación de edad específicamente para los parientes de las mujeres era del 22% y 44% respectivamente. Aproximadamente la mitad de los individuos con tiroiditis de Hashimoto eran eutiroideos y era más probable que tuvieran otros anticuerpos y una historia familar positiva que aquéllos que eran hipotoroideos o que no tenían enfermedad tiroidea. Los análisis genéticos revelaron una duplicación en el incrementon en DQA1*0501-DQB1*0201 entre los haplotipos Hashimoto y no Hashimoto. Estos hallazgos sugirieron que la tiroiditis de Hashimoto era una enfermedad común en familias DMID, lo cual podía ser debido en parte a genes de susceptibilidad.*

Palabras clave: *Diabetes mellitus insulino dependiente (IDDM), enfermedad tiroidea autoinmune, tiroiditis de Hashimoto, HLA-DQ, epidemiologia molecular.*

Summary

*This paper presents preliminary data regarding tlze prevalence and risk factors for autoimmune thyroid disease in IDDM probands ascertained from the Children's Hospital of Pittsburgh IDDM Registry for 1950-1965 (n = 669). Living IDDM probands who participated in the 1990 follow-up survey (n = 380) were recruited for the Familial Autoimmune and Diabetes Study. Siblings and parents were also invited to participate. To date, 255 IDDM probands and 597 parents and siblings have been evaluated. The diagnosis of autoimmune thyroid disease was based on a clinical evaluation, medical history, and laboratory determinations. Graves disease was rare in this cohort (n = 5). However, Hashimoto's thyroiditis was common among women. Prevalence rates ranged from 54% for IDDM women age <40 years to 75% for those >50 years. Corresponding age-specific estimates for female relatives were 22% and 44%, respectively. Approximately one-half of the Hashimoto's individuals were euthyroid: they were more likely to have other autoantibodies and a positive family history than those who were hypothyroid or had no thyroid disease. Genetic analyses revealed a 2-fold increase in DQA1*0501-DQB1*0201 among the Hashimoto's compared to the non-Hashimoto's haplotypes. These findings suggested that Hashimoto's thyroiditis was common in IDDM families, which may be due, in part, to common disease susceptibility genes.*

Key Words: *Insulin-dependent Diabetes mellitus (IDDM), Autoimmune thyroid disease Hashimoto's thyroiditis, HLA-DQ, Molecular epidemiology*

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Introduction

Individuals with insulin-dependent diabetes mellitus (IDDM) frequently develop multiple autoantibodies and other autoimmune diseases.[1-7] An early study reported that approximately 50% of IDDM females and 30% of IDDM males age 20-40 years had thyroid or gastric parietal cell autoantibodies.[2] Similar rates were found among healthy unrelated controls who were aged 60 years or older. Recent investigations have also revealed that IDDM individuals with other autoimmune diseases are more likely to have autoantibodies to glutamic acid decarboxylase and other pre-clinical markers for IDDM than those without autoimmune disorders.[8,9] These findings suggest that one's ability to maintain immunological self-tolerance may be lost prematurely in a sub-group of individuals with IDDM.

Autoimmune diseases have also been shown to cluster in IDDM families.[10] For example, first degree relatives of individuals with IDDM are more likely to develop autoimmune thyroid disease than individuals in the general popuiaiion. However, the causes of the occurrence of more than one autoimmune disease within an individual, or reasons for their clustering in families remain unclear. Possible explanations include linkage disequilibrium among HLA susceptibility genes and/or common environmental factors that may be related to their respective etiologies.

The currently funded Familial Autoimmune and Diabetes (FAD) Study (NIH Grant R01 DK44590, Janice S. Dorman, P.I.) is designed to evaluate the aggregation of autoimmune thyroid disease and rheumatoid arthritis in IDDM families. There are two specific aims: 1) to estimate the cumulative risks of rheumatoid arthritis and autoirnmune thyroid disease in IDDM cases, their parents and siblings, and the general population of Allegheny County, PA, and 2) to conduct an analytic epidemiologic investigation of the potential determinants (i.e., HLA haplotypes) of autoimmune thyroid disease, rheumatoid arthritis and IDDM in families. This report will present the preliminary FAD Study data regarding the prevalence and risk factors for autoimmune thyroid disease in the IDDM families.

Material and rnethods

A. Study population

The FAD Study is based on the Children's Hospital of Pittsburgh (CHP) IDDM Registry for 1950 - 1965 (n = 669). This cohort was originally defined in 1981 for a study of IDDM mortality. Individuals were eligible if they were: 1) on insulin therapy at hospital discharge, 2) less than age 17 years at disease onset, 3) diagnosed between 1950 and 1965, and 4) seen at CHP for a diabetes evaluation within one year of IDDM onset. Approximately 50% of the population is female and 95% is Caucasian. During 1990, all registered cases were recontacted to update the reproductive data regarding the number of pregnancies, their outcomes and the diabetes status of the offspring. Self-report information concerning the presence of other autoimmune diseases in the proband, his/her parents, siblings and children was also collected. These data were obtained for 87% (n=579) of the cohort.

Living IDDM cases who completed the 1990 survey were recontacted in 1993 and invited to participate in the FAD Study (n=380). Those who agreed received a clinical examination of autoimmune thyroid disease at our Diabetes Research Center, or they participated through an out-of-town evaluation or a home visit. Parents and siblings of participating IDDM cases were also recruited; they received identical clinical and laboratory evaluations. To date, 262 IDDM probands and 561 siblings and parents have participated. In addition, 57 control families representing the general population of Allegheny County, PA have been evaluated.

B. Clinical and Laboratory Evaluations

The diagnosis of autoimmune thyroid disease was based on the clinical evaluation, medical history, assessment of signs and symptoms and laboratory determinations, including levels of free thyroxine (FT4), thyrotropin (TSH) and thyroid

autoantibodies (i.e., thyroid peroxidase (TpAb), thyroglobulin (TgAb) and TSH receptor antibodies (TRAb)). Hashirnoto's thyroiditis with hypothyroidism was defined using a combination of four criteria: 1) the presence of high titres of TpAb or TgAb (> 10 U/ml), 2) elevated levels of TSH (>5 mU/l) in the absence of rnedications, 3) a positive rnedical history, andlor 4) a positive clinical examination. The latter two criteria included a previous diagnosis of Hashimoto's thyroiditis, current usage of thyroid medication, a palpable goiter, andlor signs and symptorns of Hashirnoto's thyroiditis. Euthyroid Hashimoto' s thyroiditis was similarly defined, except that TSH values were normal in the absence of thyroid horrnone rnedication. Graves disease was defined by high titres of TRAb (>10%), undetectable TSH (<0.1 mU/L), high T3 (>250 mg/dl), andlor a positive rnedical history or clinical examination. This included a previous diagnosis of Graves disease, current usage of antithyroid medication or previous therapy, andlor signs and symptorns of Graves disease.

In addition to the clinical examination for autoirnrnune diseases, all participants were evaluated for possible risk factors for these disorders. This included an assessrnent of glycosylated hemoglobin, lipid levels, rheumatoid factor and the presence of anti-nuclear autoantibodies (ANA). Participants also completed several questionnaires to assess past medical history, lifestyle habits, gynecological and reproductive history, socioeconornic characteristics, psychosocial factors and an extended family history of autoimmune diseases.

HLA-DQAl and DQBl rnolecular typing was performed on DNA extracted from peripheral blood lymphocytes using standardized laboratory methods. The second exons encoding the first polyrnorphic domains of the HLA-DQAl and DQBl genes were selectively PCR arnplified using specific flanking primers, Arnplitaq DNA polyrnerase and a thermocycler from Perkin Elrner Cetus, according to previously published methods.[11,12] Eight DQA 1 alleles and 14 DQBl alleles were evaluated. The nornenclature of the World Health Organization Nomenclature Comrnittee for Factors of the HLA System was utilized.[13]

C. Statistical analyses

Chi square tests and analyses of variance were ernployed to evaluate univariate association between thyroid disease status and discrete and continuous variables, respectively.[14] Haplotype frequencies were obtained by gene counting. Cornparisons between Hashirnotot's thyroiditis and non-Hashimoto's thyroiditis haplotypes were rnade using the Chi square test, with Yates correction.

Results

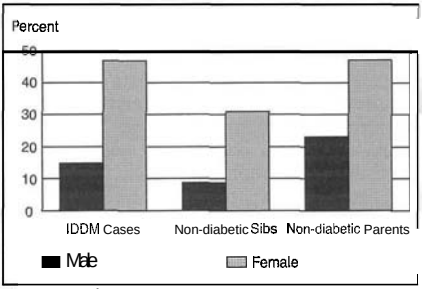
A. Demographic characteristics of FAD *study* participants

Forty-nine percent of the IDDM participants were male (n=137) and 51% were fernale (n=142). The average age at follow-up was 41.4 years and 42.0 years for males and fernales, respectively. Males were diagnosed at a mean age of 7.4 years and females developed IDDM at a mean of 8.0 years. The mean IDDM duration for males and fernales was 34.1 years and 33.9 years, respectively.

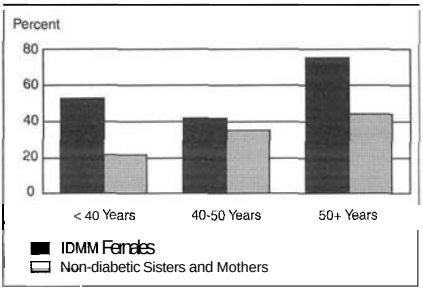
Non-diabetic fathers (n=79) were on average 72 years of age, and the mean age of the rnothers (n=128) was 69 years. Non-diabetic brothers (n=126) and sisters (n=172) had rnean ages of 41 years and 42 years, respectively.

B. Prevalence of Autoimmune *Thyroid Disease*

Graves disease was rarely obsewed in the IDDM families (n=5). However, Hashirnoto's thyroiditis was cornrnon in the FAD Study cohort.[15] The prevalence of Hashimoto's thyroiditis arnong IDDM cases, non-diabetic siblings and non-diabetic parents was consistently higher for women than for rnen (Figure 1). Among IDDM women, the prevalence of Hashimoto's thyroiditis increased with age, frorn 54% for individuals less than age 40 years to 75% for those greater than age 50 years (Figure 2). A similar pattern was obsewed for nondiabetic sisters and rnothers (Figure 2), but not for non-diabetic brothers or fathers (data not shown).



Fiiure 1 Prevalence of Hashimoto's Thyroiditis Among IDDM Cases and Non-diabetic Parents and Sibinos. Males (Black bars): Females (Shaded bars).



Figute 2 Prevalence of Hashimoto's Thyroiditis by Age in Women. IDDM (Black bars); Non-diabetic (Shaded bars).

Among the IDDM cases, non-diabetic siblings and non-diabetic parents with Hashimoto's thyroiditis, 48%, 49% and 60%, respectively, were euthyroid (i.e., high antibody titres, normal TSH, negative history and normal exam). The remaining Hashimoto's thyroiditis cases were hypothyroid (i.e., high antibody titres, normal/elevated TSH, positive history and/or positive exam).

The characteristics of individuals with hypothyroid Hashimoto's thyroiditis were compared to those who were euthyroid Hashimoto's, as well as those who had no autoimmune thyroid disease to determine if there were demographic or immunologic factors that distinguished individuals with thyroid dysfunction from those with autoantibodies, but no physiological evidence of the disease. As illustrated in table I for IDDM cases, these groups were similar with regard to age at interview, age of IDDM onset and duration of IDDM. However, gender, high antinuclear antibody (ANA) titres and a positive family history of Hashimoto's thyroiditis varied significantly among the three groups. Interestingly, the euthyroid group was most likely to have evidence of other autoantibodies and a family history of the disease. Similar descriptive results were obsewed for non-diabetic parents and siblings (Table II). However, arnong the relatives, those with Hashimoto's thyroiditis were significantly older than individuals with no thyroid disease. These data suggest that immunological abnormalities rnay be greater among Hashimoto's individuals with normal thyroid function compared to those who are hypothyroid.

Cuadro I. **Characteristics** of **IDDM** Cases With and **Without Hashimoto's Thyroiditis**

Characterisitic	Hypothyroid Hashimoto's (n=37)	Euthyroid Hashimoto's (n=34)	No Thyroid Oisease (n= 158)
Female	78.4%	73.5%	38.0%
Mean Age at Interview	42.4 yrs	41.0 yrs	40.9 yrs
Mean Ageat IDDM	7.1 yrs	9.1 yrs	7.4 yrs
Mean Durationof IDDM	35.2 yrs	31.9 yrs	33.5 yrs
ANA Titres 2 160	11.4%	21.2%	7.2%*
Family History of HT	54.5%	85.7%	44.6%*

*p<0.05 among the three groups

Table II. **Characteristics of Non-diabetic Siblings and** Parents With and **Without** Hashimoto's *Thyroiditis*

Characterisitic	Hypothymid Hashimoto's (n=46)	Euthyroid Hashimoto's (n=57)	No Thyroid Oisease (n= 256)
Female	78.3%	78.9%	52.3% *
Mean Ageat Interview	54.2 yrs	60.4 yrs	51.1 yrs*
ANA Titres 2 160	0.0%	16.7%	3.5%

*p<0 05 among the three groups

C. Genetic Analysis: DQA*1-DQB1* Haplotype *Distribution* and Hashimoto's *Thyroiditis*

To begin to investigate the immunogenetics of Hashimoto's thyroiditis, families with at least one parent and one offspring with the disease were identified.[16] A total of 25 parent offspring Hashimoto's thyroiditis families have fully participated and have been typed for HLA DQA1 and

DQB1 alleles. For these analyses, parental Hashimoto's haplotypes were defined as those occurring in affected individuals, and non-Hashimoto's thyroiditis haplotypes represented those carried only by individuals with no autoimmnune thyroid disease. Despite the low prevalence of Hashimoto' s thyroiditis among men in the total FAD Study cohori (15%), 37% of the affected parents were fathers, and 63% were mothers. The mean ages of the Hashimoto's and non-Hashimoto's parents were 67 years and 69 years, respectively. In ten families, there were multiple offspring with Hashimoto's thyroiditis. The total number of affected offspring was 39. Sixty-nine percent of those with Hashimoto's thyroiditis were female, 31% were male. Hashimoto's and non-Hashimoto's offspring were 40 years and 38 years, respectively. Among the Hashimoto's offspring, 59% of the females and 75% of the males also had IDDM. Interestingly, the prevalence of multiplex IDDM families was higher in the group of Hashimoto's families than in the total FAD cohori (20% vs. 12%).

Since there was at least one affected IDDM offspring in each family, there was an enrichment of DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302 haplotypes in this population, which are in linkage disequilibrium with HLA-DR3 and DR4, respectively. Interestingly, the distribution of DQAI*0501-DQB1*0201 haplotypes differed from that for DQA1*0301-DQB1*0302 haplotypes with respect to Hashimoto's thyroiditis. As illustrated in figure 3, there was a two-fold increase in the frequency of DQA1*0501-DQB1*0201 in the Hashimoto's compared to the non-Hashimoto's group (23% vs. 9%, respectively). However, no difference in prevalence was obsewed for DQAI *0301-DQB 1 *0302 (21 % vs. 18%, respectively). Othercommonhaplotypesin theHashimoto'sgroup were DQA1*0101-DQB1*0501 (11% vs. 9%), DQAI *0501-DQB1*0301 (10% vs. 0%) and DQA1*0102-DQB1*0602 (9% vs. 0%). All of the 11 parents who carried DQAI*0501-DQB1*0201 transmitted the haplotype to a Hashimoto's thyroiditis offspring. However, only six of the eight affected offspring of parents with DQA*0301-DQB1*0302 inherited this haplotype from their affected mother or father.

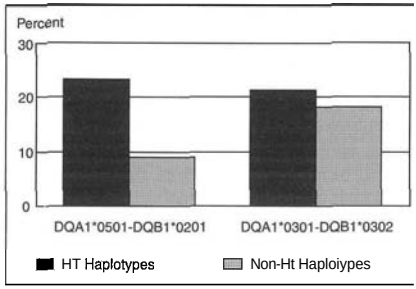


Fig.re 3 n-A-DQA1-DQB1 Hap otypes in Parent-Offspring hasn moto's Thyroiditis (nT) Fami es nT haplotypes (Black bars) Non-nT haplotypes (Shaded bars).

Although there were very few individuals with Graves disease in the FAD Study cohort, four of the five affected persons were mothers. Among the four who were HLA typed, three (75%) carried DQA1*0301-DQB1*0302. No Graves disease individual carried DQAI*0501 DQB1*0201. These findings are consistent with the literature and suppori previous findings concerning the importance of DQAI*0501-DQB1*0201 as a susceptibility haplotype for both Hashimoto's thyroiditis and IDDM. Alternatively, DQA1*0301-DQB1'0302 may be associated with Graves disease.

Discussion

Preliminary data from the FAD Study confirmed the high prevalence of autoimmune thyroid disease among individuals with IDDM and their first degree relatives. Although Graves disease was rarely obsewed in our IDDM families, Hashimoto's thyroiditis was common, pariicularly among women. The prevalence of Hashimoto's thyroiditis among IDDM females (47%) was equal to that obsewed among their non-diabetic mothers (47%). Moreover, the prevalence of Hashimoto'sthyroiditisincreased with age in both IDDM and non-diabetic women, with rates consistently higher among those with IDDM. However, the rate of Hashimoto's thyroiditis among IDDM men was significantly lower than for that IDDM women (9% vs. 47%, $p<0.05$). The prevalence among IDDM men was also similar to

rates observed for non-diabetic brothers and fathers, and no increase with older age was observed among the men. These data were similar to those reported by an earlier study for Caucasian subjects[3] and suggest that some IDDM individuals, particularly women, may prematurely exhibit signs and/or symptoms of the normal aging process.

Approximately one-half of the Hashimoto's individuals in the FAD Study cohort were euthyroid, regardless of diabetes status (i.e., high antibody titres, normal TSH, negative history and normal exam), and thus had no signs and symptoms of thyroid disease. Whether these individuals are at high risk for developing thyroid dysfunction was not clear. Prospective follow up of such cases may provide important insight regarding the determinants of thyroid dysfunction associated with Hashimoto's thyroiditis. Interestingly, our cross-sectional comparisons revealed that euthyroid Hashimoto's individuals were more likely to have a positive family history of Hashimoto's thyroiditis and evidence of antinuclear antibodies than those who had hypothyroid Hashimoto's thyroiditis. These data emphasize the importance of continued follow-up of euthyroid individuals.

With regards to the immunogenetic analyses of autoimmune thyroid disease in IDDM families, our data suggested that susceptibility to Hashimoto's thyroiditis was associated to DQA1*0501-DQB1*0201, which is in linkage disequilibrium with DR3. This was consistent with other molecular serological studies in the literature.[17] DQA1*0301-DQB1*0302, however, did not appear to be associated with Hashimoto' s thyroiditis in IDDM families, but may instead predispose to Graves disease. Given our small sample of Graves cases, further data are required to confirm the findings.

The FAD Study will, therefore, provide important information regarding the risk and potential determinants of the familial aggregation of autoimmune thyroid disease in IDDM families. Follow-up of relatives at high risk will help elucidate etiologic relationships and permit an evaluation of the natural history of the disease.

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