PREVALENCE AND CORRELATION OF ISLET AUTOANTIBODIES (ICA, IA-2, GAD-65) AND THYROID ANTIBODIES IN FIRST-DEGREE RELATIVES OF IDDM PATIENTS

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Objective

Type I diabetes mellitus is thought to result from autoimmune destruction of pancreatic β-cells and is frequently associated with organ- specific autoimmune diseases, including autoimmune thyroid disease, pernicious anemia and idiopathic Addison's disease.

The aim of the present study was to determine the significance of thyroid, adrenal and gastric-parietal cell antibodies in 1st degree relatives of type I diabetic patients who are at risk for diabetes (islet autoantibody positivity).

Material and Methods

In this study, 842 1st degree relatives of type I diabetes mellitus (mean age 14.6 ± 8.7 yrs) participated.
All of these subjects participated in the Giessen - Bad Oeynhausen Family Study. In all cases, serum obtained at study entry was analyzed for ICA, IA-2ic, GAD 65 antibodies as well as for thyroid (TG/TPO), adrenal (AD-Ab) and gastric-parietal cell (GPC-Ab) antibodies.
Antibody status of these subjects at study entry is shown in Table 1.

Study Population (n= 842)	Number of Subjects (%)
• ICA pos.	67 (7.9)
• IA-2ic pos.	57 (6.9)
• GAD65 Ab pos.	84 (9.9)
• Thyroid Ab pos. (TG and TPO)	28 (3.3)
• Adrenal Ab pos.	3 (0.4)
• GPC Ab pos.	17 (2.0)
• Thyroid & Adrenal Ab pos.	0 (0)
• Thyroid & GPC Ab pos.	0 (0)
• Adrenal & GPC Ab pos.	0 (0)

Table 1: Antibody (Ab) characteristics of the study population (n=842). Data are given as absolute numbers (%).

Islet cell antibodies: Islet cell antibodies (ICA) were determined by indirect immunofluorescence technique using cryostat sections of human pancreas as substrate. This assay is regularly tested in the IDW proficiency workshop series on standardization of the ICA assay. Titres have been converted to Juvenile Diabetes Foundation (JDF) units using a JDF standard reference serum. The detection limit of the assay is 5 JDF units.

GAD 65 antibody assay: GAD 65 antibodies were detected in a radioligand GAD 65 assay using as tracer recombinant, in vitro translated, human [35S]- methionin-labelled GAD 65. The cut-off index level for GAD 65 antibody positivity was determined from 150 healthy control subjects. Sera with GAD 65 antibody index values above the mean index plus three times the standard deviation were regarded as positive. This assay has been evaluated in the first IDW proficiency workshop on GADA, all samples were tested in duplicate.

IA-2ic antibody assay: Autoantibodies to the intracytoplasmic domain of IA-2 (anti-IA-2ic) were determined using radiolabelled recombinant antigens in a 96-well assay format. Recombinant autoantigens were produced by coupled in vitro trascription and translation of human IA-2ic cDNA. To achieve high specificity the cut off for antibody positivity was set at mean + 4 SD of antibody levels in 100 normal controls. In the combined IDW autoantibody workshop this assay had a diagnostic sensitivity of 73% and a specificity of 96% for type I diabetes.

Thyroid autoantibodies were determined with a commercially available enzyme-linked immunosorbent assay, using recombinant human TG or TPO as substrate.

Adrenal and gastric-parietal cell antibodies were detected by indirect immunofluorescence on cryostat sections of rat as substrate.

Statistical analysis

Correlations were calculated according to Spearman rank correlation coefficient, analysis of variance (ANOVA) and Kruskal-Wallis analysis by ranks was applied and multiple range analysis according to Scheffe's method was used when appropriate. A p value of less than 0.05 was considered to be significant.

Results

- A statistically significant correlation was observed between ICA and thyroid antibodies ($p<0.05$) Fig.1

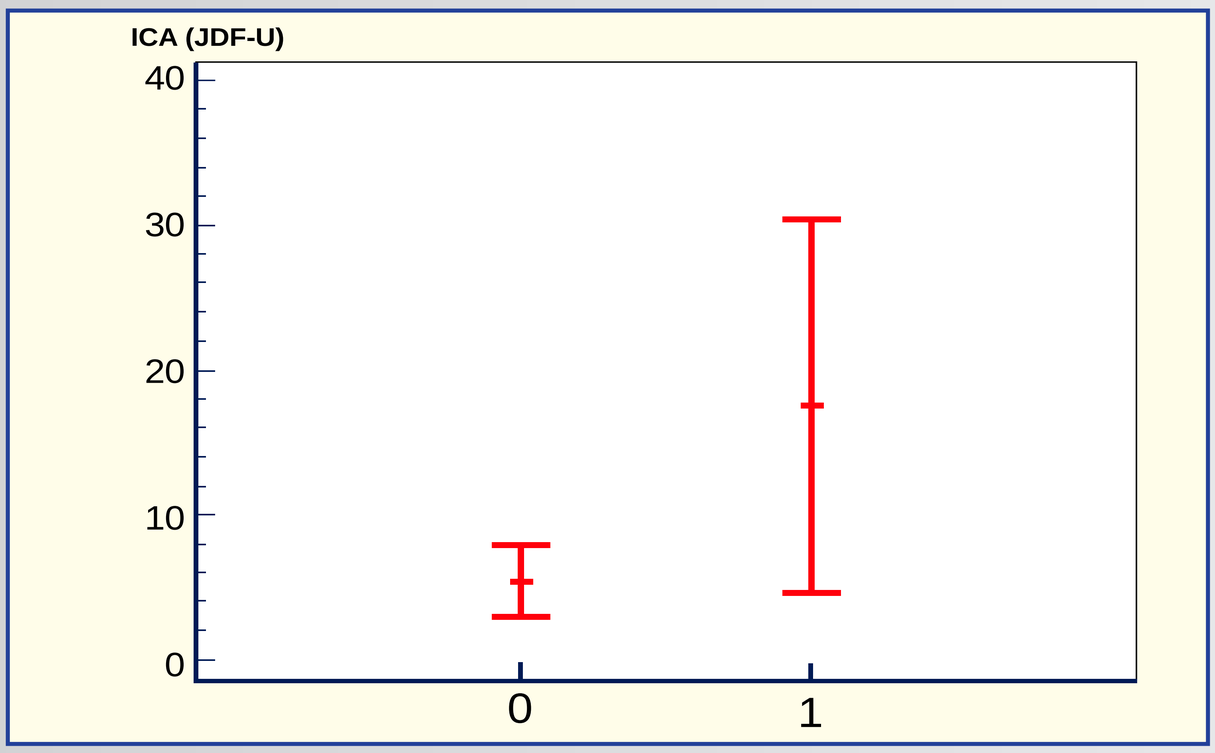

Figure 1: 95 percent confidence interval for ICA means depending on TG / TPO antibody positivity

- A significant association was also seen between IA-2ic and thyroid antibodies ($p<0.01$) Fig. 2. Higher levels of IA-2ic Ab were observed in subjects with TPO-Ab positivity in comparison to those tested negative for TPO-Ab.

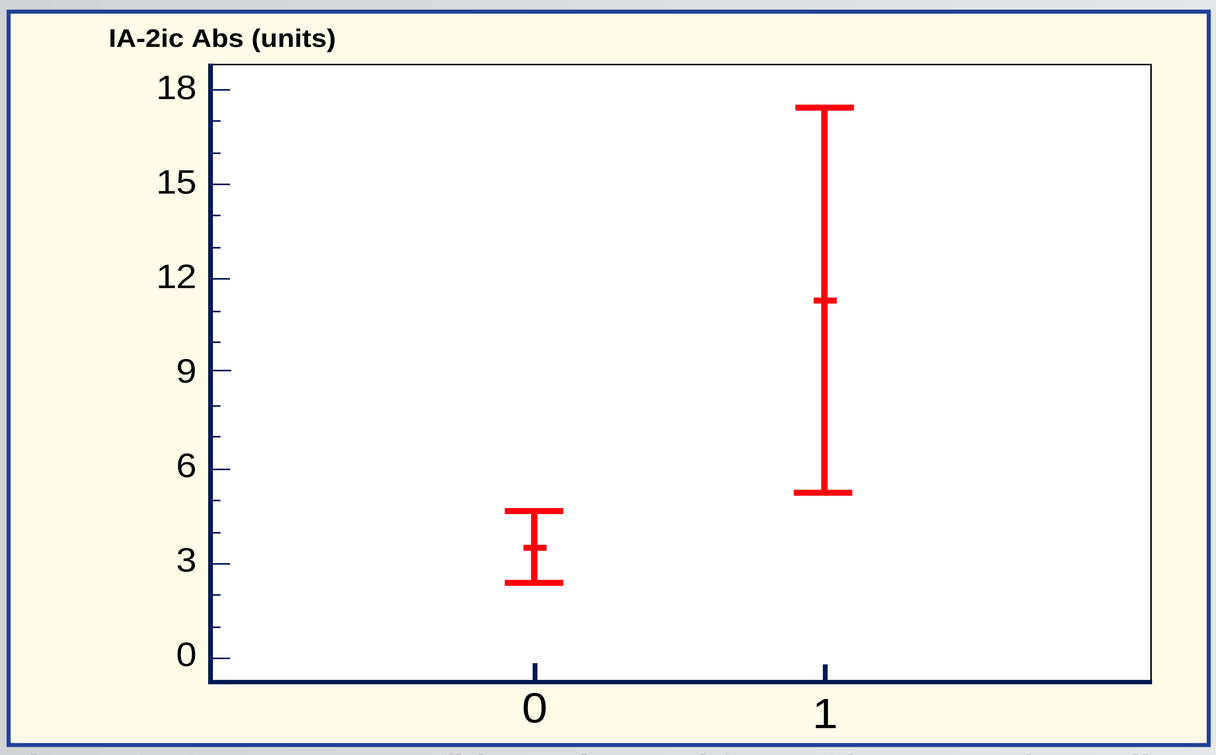

Figure 2: 95 percent confidence interval for IA-2ic means depending on TG / TPO antibody positivity

- A positive trend but not significant was also observed between GAD 65 and thyroid antibodies.
- There was no significant association of any antibody (ICA / IA-2 / GAD65) with adrenal and gastric-parietal cell antibodies.

Conclusion

Our observations indicate that humoral autoimmunity particularly against ICA and IA-2ic may be determined by the same factors as those controlling susceptibility to thyroid autoimmunity.
Islet autoantibodies may predict susceptibility to thyroid disease not only in diabetic patients but also in their relatives who are at high risk for type I diabetes.