

## Original Article

# Association between IgE-mediated allergies and diabetes mellitus type 1 in children and adolescents

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**Background:** Type 1 diabetes mellitus (T1DM) is characterized by an immunological reaction that is dominated by type-1 T helper (Th1) cells, whereas immunoglobulin E (IgE)-mediated allergies are associated with Th2 cell. According to the Th1/Th2-hypothesis, the immune system is said to either develop into the direction of Th1 or Th2 cells. This would mean that a child developing T1DM is unlikely to develop an IgE-mediated allergy and vice versa.

**Objective:** The aim of the study was to investigate the association between the prevalence of T1DM and IgE-mediated allergies.

**Methods:** We designed a prospective case control study with 94 children and adolescents with T1DM and 188 age- and sex-matched control children. The basis of our investigations was a questionnaire concerning the family and children's history as to the presence of IgE-mediated allergies. Moreover, the following blood investigations were done: total serum IgE, specific IgE antibodies to major inhalant allergens, and a multiplex cytokine analysis measuring levels of specific cytokines representing either Th1- or Th2-cytokines.

**Results:** Children with T1DM reported the presence of IgE-mediated allergies significantly more often than children of the control group. Children with T1DM had significantly higher tumor necrosis factor alpha (TNF $\alpha$ ) levels than healthy controls. Levels of interleukin-2 (IL-2) and IL-6 were higher in the groups of children with the presence of a personal history of allergies, regardless of the presence of T1DM.

**Conclusions:** Our results suggest that T1DM is associated with a higher risk of a self-reported presence of IgE-mediated allergies and that the Th1/Th2-hypothesis may be an oversimplification.

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Type 1 diabetes mellitus (T1DM) and immunoglobulin E-mediated allergies belong to the most common chronic diseases in children and adolescents that

show increasing incidence rates. It is well known that both diseases have a multifactorial pathogenesis that is influenced by genetic, environmental, and

immunological factors. T helper (Th) cells play an important role in the development of both diseases. There are two main subtypes of Th cells which have been reported in the past, called Th1 and Th2 cells (1). T1DM is caused by an autoimmune process which leads to a progressive destruction of pancreatic beta cells (2). From the immunological point of view it is assumed that this process is dominated by the Th1 subfraction (3). In contrast IgE-mediated allergies, for example, allergic asthma, allergic rhinitis, or allergic urticaria, are thought to be associated with Th2 cells. Up to the present, the hypothesis that Th1 and Th2 cells are antagonists suppressing each other is still valid. For clinical practice this would mean that an individual developing T1DM is unlikely to develop an IgE-mediated allergy and vice versa (4).

In the literature, the results of the few studies that investigated the coexistence of allergies and T1DM are very controversial. Some of them showed a negative association between the two diseases (5–9), whereas others detected a positive association (10, 11) or no relation at all (12–14). All these studies show many differences in their methodology; therefore it is difficult to compare them.

According to the existing controversial data, the knowledge of both diseases increasing in incidence rates and clinical experience that has indicated that allergic symptoms seem to be quite common in children and adolescents with T1DM, we decided to implement a case control study. The aim of this study was to investigate the association between the prevalence of T1DM and IgE-mediated allergies, using not only information about the presence of clinical symptoms but also objective parameters such as investigating total-IgE and specific cytokine levels in blood samples. Cytokines play an important role in inflammatory processes and therefore also in inducing beta-cell damage in patients with T1DM (15). There are only a few studies with respectively low numbers of cases and conflicting results that investigated levels of cytokines in patients with T1DM. A study from Turkey, for example, investigated serum concentrations of tumor necrosis factor alpha (TNF $\alpha$ ), interleukin-6 (IL-6), and IL-2 in patients with T1DM compared with healthy controls. They found that TNF $\alpha$  levels were elevated in the group of T1DM patients, whereas both IL-2 and IL-6 were lower in this group (16). Other studies found an increase in serum IL-6 levels in patients with T1DM (15, 17), whereas others found no difference (18). Results of measuring TNF $\alpha$  levels are also reported to be increased (15, 19) in patients with diabetes, decreased (20) or not different either (21, 22). Investigations of serum levels of IL-10 in diabetic patients are even rarer. In 2013 Kikodze et al. found decreased levels of IL-10 in patients with T1DM compared with healthy controls (23). Knowing that chronic

inflammation plays a role in the pathogenesis of IgE-mediated allergies, cytokines have been investigated in this context, too. Wong et al., for example, found that levels of IL-10 and IL-13 were significantly higher in patients with allergic asthma than in healthy controls (24). Furthermore they found higher levels of IL-6 and IL-17 in allergic patients, although these differences were not statistically significant. In contrast, in 2013, Stelmaszczyk-Emmel et al. concluded that plasma concentrations of both, Th1 and Th2, as well as regulatory cytokines are quite low in allergic children (25). The basis for our scientific analyses is the following hypothesis: Children and adolescents with T1DM may suffer from an IgE-mediated allergy more often than healthy children and adolescents of the same age, same region, and same population background. The following questions are used to verify this hypothesis: Are there differences in the frequency of allergic diseases like allergic asthma, allergic rhinitis, allergic urticaria, or allergic shock between children and adolescents with T1DM and their healthy controls? Are there differences in the frequency of allergic diseases in the family history between the cases and controls? Are the total IgE values in the serum of children and adolescents with T1DM higher than in the serum of their controls? Are there any differences in the cytokine profile between the two groups?

## Methods

### Design and subjects

We designed a prospective case control study, using cases of diabetes mellitus type 1 and healthy control children. The study population consisted of 94 children and adolescents with diabetes mellitus type 1, aged between 3 and 21 yr, who are all seen by a diabetes specialist at the Hospital for Children and Adolescents of the University of Leipzig, on a regular basis. There were 59 boys and 35 girls. The median age was 12 yr. The control population consisted of 188 age- and sex-matched non-diabetic children and adolescents. They were recruited by the Leipzig Research Center for Civilization Diseases (LIFE) (26). There were 118 boys and 70 girls. The median age was 12 yr (minimum 3 yr, maximum 18 yr). Baseline data concerning both the case and the healthy control sample are shown in Table 1. Specific information about the children and adolescents with T1DM are shown in Table 2. The study was approved by the Ethical Committee of the University of Leipzig (reference number: 035-10-09112009). The initially planned skin-prick tests were not approved by the Ethical Committee. At least one parent of each participant of the study and the participant her/himself (if aged 12 yr or older) signed an informed consent.

Table 1. Baseline data of the children with T1DM and the healthy control children

|                         | Type 1 diabetes, n = 94 | Healthy controls, n = 188 | p Value |
|-------------------------|-------------------------|---------------------------|---------|
| Age in years, mean      | 12.12 (4.55)            | 12.07 (3.97)              | NS      |
| Gender, male, count (%) | 59 (63%)                | 118 (63%)                 | NS      |
| BMI-SDS, mean (SD)      | 0.52 (0.88)             | 0.02 (1.16)               | <0.001  |

BMI-SDS, body mass index standard deviation score; NS, not significant; SD, standard deviation; T1DM, type 1 diabetes mellitus.

Table 2. Specific information of the children with T1DM

|                                                         | Type 1 diabetes, n = 94 |
|---------------------------------------------------------|-------------------------|
| Age at onset of T1DM, median (min/max)                  | 7.5 (0/16)              |
| Years since diagnosis, median (min/max)                 | 3 (0/14)                |
| HbA1c level in mmol/mol Hb, median (min/max)            | 66.1 (38.8/128.4)       |
| HbA1c level in %, median (min/max)                      | 8.2 (5.7/13.9)          |
| Cumulative HbA1c level in mmol/mol Hb, median (min/max) | 65.0 (43.2/128.4)       |
| Cumulative HbA1c level in %, median (min/max)           | 8.1 (6.1/13.9)          |

HbA1c, hemoglobin A1c; cumulative HbA1c, average of HbA1c levels of the last year; min, minimum; max, maximum; T1DM, type 1 diabetes mellitus.

## Questionnaire

Following the requirements of the International Study of Asthma and Allergies in Childhood (ISAAC) (27) the parents of all children were asked to answer a structured questionnaire. It contained standardized and evaluated questions concerning the family history as to the presence of allergic rhinitis, allergic asthma, allergic urticaria, or the occurrence of an allergic shock in the past and the children's medical history as to the presence of allergic rhinitis, allergic asthma, allergic urticaria, or an allergic shock in the past.

## Laboratory measurements

Levels of total serum IgE and a SX1 (inhalant screen) screen were analyzed using the ImmunoCAP® system. The SX1 screen was used to investigate the presence of IgE antibodies against a mixture of the eight major inhalant allergens, namely house dust mite, cat scales, dog scales, rye, timothy grass, cladosporium herbarum, birch tree, and mugwort. For interpretation of the results we used the reference values recommended by the manufacturer (Phadia AB, Uppsala, Sweden, 2013). The intra-assay coefficient of variation was 3% for both assays. The inter-assay coefficient of variation was between 4 and 7%, depending on the IgE value. Furthermore the levels of four cytokines were measured in the blood samples by a multiplex cytokine analysis: TNF $\alpha$  and IL-2 as representatives for Th1 and IL-6 and IL-10 for Th2 cytokines. This investigation ran

parallel for both the case and control group to be able to submit valid statements. The intra-assay coefficient of variation for this assay was between 1.6% (IL-10) and 2.6% (TNF $\alpha$ ), the inter-assay coefficient of variation was between 6.3% (IL-2) and 18.3% (IL-6). The hemoglobin A1c (HbA1c) level was measured in the group with T1DM to define the degree of metabolic control. The reference value for the HbA1c level was 4.8–5.9%, respectively 29–42 mmol/mol Hb (Roche, Rotkreuz, Schweiz).

## Data analysis

The study population was characterized using common descriptive statistics. Odds ratios were calculated for T1DM vs. non-T1DM. Confidence intervals were computed using the normal approximation to log-odds. Data was further grouped by family history concerning allergies (positive/negative) and T1DM (yes/no). Risks and respective credible intervals were estimated using a Bayesian approach: generalized linear models including interaction term with normal priors on the covariate coefficients and flat priors on the intercept were applied. The respective p values were determined by simulation. For TNF $\alpha$ , IL-2, IL-6, and IL-10 below limits of detection uniformly distributed values between 0 and limit of detection were generated. Levels of TNF $\alpha$ , IL-2, IL-6, and IL-10 were compared by a analysis of variance (ANOVA) with Tukey's HSD post-hoc analyses. If p was <0.05 the value was considered as significant.

## Results

The study included 94 children and adolescents with T1DM and 188 healthy controls. Table 1 shows baseline data of these two groups. There were no statistically significant differences in terms of mean age [T1DM: 12.12 yr ( $\pm$ 4.55), no T1DM: 12.07 yr ( $\pm$ 3.97)] and distribution of sex (63% males and 37% females, p = 1) between the two groups. The mean body mass index-standard deviation score (BMI-SDS) was significantly higher (p < 0.001) in the group of children with T1DM than in the control group, which has been reported before (28, 29). Table 2 summarizes specific information of the group of children and adolescents with T1DM; the median age at onset of T1DM was 7.5 yr (range 0–16 yr) and the median duration of diabetes was 3 yr (range 0–14 yr). The mean cumulative HbA1c level, which is the average of all HbA1c levels of the last year, was 8.1% (range 6.1–13.9%) respectively 65 mmol/mol Hb (range 43.2–128.4), including also children at manifestation of their diabetes. The frequency of various allergic symptoms in the personal and family history in children with T1DM and in the healthy control children

Table 3. Frequency of allergic symptoms in children with T1DM and in healthy control children

| Allergic symptoms                 | Type 1 diabetes,<br>n = 94 | Healthy controls,<br>n = 188 | p Value      | Odds ratio (95% CI)  |
|-----------------------------------|----------------------------|------------------------------|--------------|----------------------|
| Personal history                  |                            |                              |              |                      |
| Allergic rhinitis                 | 10 (10.6%)                 | 11 (5.9%)                    | 0.149        | 1.916 (0.783–4.687)  |
| <b>Allergic urticaria</b>         | 22 (23.4%)                 | 25 (13.3%)                   | <b>0.032</b> | 1.992 (1.054–3.765)  |
| Allergic asthma                   | 5 (5.3%)                   | 7 (3.7%)                     | 0.542        | 1.453 (0.448–4.706)  |
| Allergic shock                    | 1 (1.1%)                   | 2 (1.1%)                     | 1.000        | 1.000 (0.090–11.171) |
| <b>Positive personal history*</b> | 31 (33.0%)                 | 39 (20.7%)                   | <b>0.026</b> | 1.880 (1.078–3.278)  |
| Family history                    |                            |                              |              |                      |
| Allergic rhinitis                 | 35 (37.2%)                 | 63 (33.5%)                   | 0.536        | 1.177 (0.702–1.972)  |
| Allergic asthma                   | 20 (21.3%)                 | 32 (17%)                     | 0.385        | 1.318 (0.706–2.458)  |
| Allergic shock                    | 7 (7.4%)                   | 5 (2.7%)                     | 0.113        | 2.945 (0.909–9.542)  |
| Positive family history†          | 39 (41.5%)                 | 73 (38.8%)                   | 0.667        | 1.117 (0.675–1.850)  |

CI, confidence interval; T1DM, type 1 diabetes mellitus.

Values < 0.05 were considered as significant and printed in bold.

\*At least one of the four aspects allergic asthma, allergic rhinitis, allergic urticaria, or allergic shock is positive.

†At least one of the three aspects allergic asthma, allergic rhinitis, or allergic shock is positive.

is shown in Table 3. Children with T1DM had a significantly higher risk for a positive personal history for allergic symptoms in general than children of the control group; 33% of the children with T1DM, but only 20.7% of the children in the control group reported allergic symptoms in their personal history ( $p = 0.026$ ). Considering each symptom by itself, the odds ratio were significantly different from 1 ( $p = 0.032$ ) in the prevalence of allergic urticaria between the two groups. The odds of a positive personal history for allergic urticaria were nearly twofold higher in the case than in the control group. Adjustment for age, sex, and BMI-SDS did not influence this result and therefore was not listed separately in this table. Although the prevalence of allergic rhinitis and allergic asthma was almost twice as high and of allergic asthma 1.5 times higher among T1DM patients compared with healthy controls, this was not statistically significant, probably caused by the low prevalence rates respectively low number of subjects. There was no statistically significant difference at all in regards to the prevalence of allergic shock in the personal history between the two groups ( $p = 1.0$ ). Considering the family history there was a trend of a higher prevalence of allergic symptoms in general, allergic rhinitis, allergic asthma, and allergic shock in the group of children with T1DM, too, but the differences were not statistically significant either. Results of the laboratory investigations in the group of children with T1DM and the group of healthy children are shown in Table 4. There were no considerable differences in terms of increased total IgE ( $p = 0.572$ ) levels or positive inhalant screen ( $p = 0.121$ ) between the two groups. Combining results of the medical history and the laboratory measurements by considering the prevalence of both, positive personal history for allergic symptoms in general and increased total IgE levels, there was again a trend, but not a

significant difference, of a higher prevalence in the case group than in the control group ( $p = 0.238$ ). There was also no significant difference between the prevalences of each symptom by itself plus increased total IgE levels. Adjustment for age, sex, and BMI-SDS did not influence this result and was therefore not listed separately in this table either. The results of the multiplex cytokine analysis are also shown in this table. 26.6% of the children in the group with T1DM, but only 5.1% of the children in the healthy control group had increased ( $>10$  pg/mL) TNF $\alpha$  values. This difference was statistically significant ( $p = <0.001$ ). There were no statistically significant differences between the two groups regarding the proportions of increased values of IL-2 ( $p = 1.000$ ), IL-6 ( $p = 0.8845$ ), or IL-10 ( $p = 0.8572$ ). Figure 1 shows the relative proportion of children with positive personal history for allergic symptoms (Fig. 1A), with increased total IgE values (Fig. 1B), and of children with the combination of both (Fig. 1C) in four groups referring to the two items presence of T1DM and family history for allergic symptoms. The mean prevalences of the three items mentioned above were highest respectively in the group of children and adolescents with T1DM and positive family history for allergic symptoms. Comparing the two groups with positive family history for allergic symptoms the relative proportion of children with a positive personal history for allergic symptoms was significantly higher in the group of children with T1DM than in the healthy control group ( $p = 0.01448$ ; Fig. 1A). A similar difference was seen between these two groups referring to increased IgE values ( $p = 0.02539$ ; Fig. 1B). The same trend, but not statistically significant was seen between the two groups referring to the combination of positive personal history for allergic symptoms and increased total IgE values ( $p = 0.05706$ ; Fig. 1C). Children with T1DM and

Table 4. Results of the laboratory measurements in the group of children with T1DM and the group of healthy children

|                                                            | Type 1 diabetes, n = 94 |        |       | Healthy controls, n = 188 |        |        | Mean   | Lower CI | Upper CI | p Value          | Odds ratio (95% CI)    |
|------------------------------------------------------------|-------------------------|--------|-------|---------------------------|--------|--------|--------|----------|----------|------------------|------------------------|
| Total IgE increased                                        | 28 (29.8%)              | 143.76 | 89.28 | 50 (26.6%)                | 190.87 | 119.25 | 190.87 | 119.25   | 262.49   | 0.572            | 1.171 (0.677–2.025)    |
| SX1 screen positive                                        | 31 (33%)                | 10.05  | 4.82  | 80 (42.6%)                | 12.99  | 9.18   | 12.99  | 9.18     | 16.80    | 0.121            | 0.664 (0.396–1.115)    |
| Positive allergic rhinitis history and total IgE increase  | 6 (6.4%)                |        |       | 6 (3.2%)                  |        |        |        |          |          | 0.220            | 2.068 (0.648–6.594)    |
| Positive allergic asthma history and total IgE increase    | 4 (4.3%)                |        |       | 6 (3.2%)                  |        |        |        |          |          | 0.650            | 1.348 (0.371–3.898)    |
| Positive allergic urticaria history and total IgE increase | 7 (7.5%)                |        |       | 10 (5.3%)                 |        |        |        |          |          | 0.481            | 1.432 (0.527–3.891)    |
| Positive personal history* and total IgE increase          | 14 (14.9%)              |        |       | 19 (10.1%)                |        |        |        |          |          | 0.238            | 1.653 (0.783–3.489)    |
| <b>TNF<math>\alpha</math> increased†</b>                   | 34 (26.6%)              | 9.86   | 7.96  | 10 (5.1%)                 | 4.24   | 3.72   | 4.24   | 3.72     | 4.76     | <b>&lt;0.001</b> | 10.081 (3.2208–14.36)  |
| IL-2 increased†                                            | 0 (0%)                  | 1.20   | 0.86  | 4 (2.1%)                  | 1.59   | 0.98   | 1.59   | 0.98     | 2.20     | 1.000            | 0.000 (0.000–)         |
| IL-6 increased†                                            | 7 (6.9%)                | 4.01   | 0.69  | 15 (7.3%)                 | 2.55   | 1.34   | 2.55   | 1.34     | 3.77     | 0.8845           | 0.933 (0.368–2.3672)   |
| IL-10 increased†                                           | 18 (16.0%)              | 9.66   | 0.32  | 34 (15.3%)                | 6.79   | 4.18   | 6.79   | 4.18     | 9.41     | 0.8572           | 1.0588 (0.5681–1.9735) |

CI, confidence interval; IgE, immunoglobulin E; IL, interleukin; SX1, inhalant screen; T1DM, type 1 diabetes mellitus; TNF $\alpha$ , tumor necrosis factor alpha.

Values &lt; 0.05 were considered as significant and printed in bold.

\*At least one of the four aspects allergic asthma, allergic rhinitis, allergic urticaria, or allergic shock is positive.

†&gt; 10 pg/mL.

the presence of allergic symptoms in the family history had a significant higher risk for a positive personal allergy history ( $p = 0.00045$ , Fig. 1A), increased IgE values ( $p = 0.00699$ , Fig. 1B), and the combination of both ( $p = 0.01714$ , Fig. 1C) than children with only T1DM. Comparing the two groups of children without the presence of allergic symptoms in the family history, there was no significant difference in the relative proportion of children with positive personal history for allergic symptoms, with increased total IgE values or of children with the combination of both. Table 5 and Fig. 2 show the results of a variance analysis of the multiplex cytokine analysis; in the first row levels of TNF $\alpha$  (Fig. 2A) and IL-2 (Fig. 2B) representing Th1-cell cytokines and in the second row levels of IL-6 (Fig. 2C) and IL-10 (Fig. 2D) representing Th2-cell cytokines, respectively in four groups referring to the two items presence of T1DM and presence of a positive personal history for allergies in general are depicted. Figure 2A shows that children with T1DM had significant higher levels of TNF $\alpha$  than children without T1DM, regardless of the presence of a personal history for allergies ( $p < 0.001$ , respectively). Within the two groups of children with T1DM there was no statistically significant difference in regards to the mean TNF $\alpha$  values ( $p = 0.512$ ), as well as within the two groups of children without T1DM ( $p = 0.999$ ). Comparing the two groups of children with positive personal histories for allergies with the two groups of children without allergies there was no statistical difference in regards to the mean TNF $\alpha$  values, either ( $p = 0.415$ ). Figure 2B shows that children without T1DM, but the presence of a personal history for allergies had significant higher levels of IL-2 than children without allergies, regardless of the presence of T1DM ( $p_1 = 0.0039$ ,  $p_2 = 0.0192$ ). Comparing the two groups of children with the presence of a personal history for allergies (T1DM, allergy and no T1DM, allergy) with the other two groups (no allergies), levels of IL-2 were significantly higher in the group of children with a positive personal history for allergies ( $p = 0.009$ ). Comparing these groups analogously in Fig. 2C, levels of IL-6 were significantly higher in the group of children with a positive personal history for allergies ( $p = 0.0034$ ). Furthermore, children with T1DM and a positive personal history for allergies had significant higher levels of IL-6 than children without these both diseases ( $p = 0.0266$ ). Between the other groups we did not find significant differences in mean levels of IL-2 and IL-6. Mean values of IL-10 (Fig. 2D) did not differ significantly between any of the groups.

## Discussion

The results of this prospective case control study show that T1DM may be associated with a higher risk of

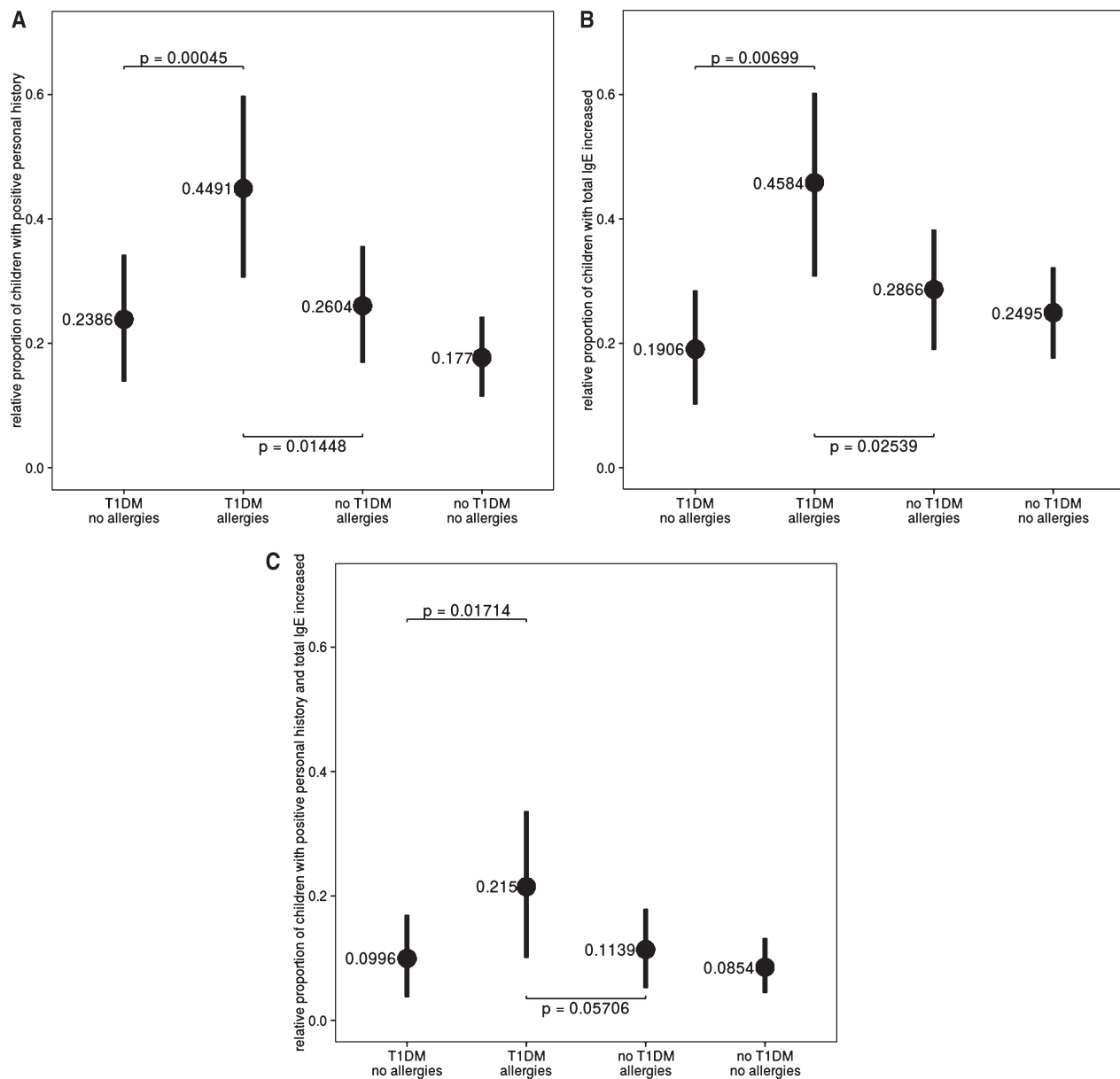


**Fig. 1.** Relative proportion of children with positive personal history for allergic symptoms (A), with increased total immunoglobulin E (IgE) (B) and of children with the combination of both (C) in four groups referring to the two items presence of type 1 diabetes mellitus (T1DM) and family history for allergic symptoms. Comparing the two groups with positive family history for allergic symptoms the relative proportion of children with a positive personal history for allergic symptoms was significantly higher in the group of children with T1DM than in the healthy control group [(A)  $p = 0.01448$ ]. A similar difference was seen between these two groups referring to increased IgE values [(B)  $p = 0.02539$ ]. The same trend, but not statistically significant was seen for the two groups referring to the combination of positive personal history for allergic symptoms and increased total IgE values [(C)  $p = 0.05706$ ].

self-reported presence of IgE-mediated allergies. This relationship confirms our assumption that the Th1/Th2 paradigm may be an oversimplification. Furthermore, our results affirm that the prevalences of both, atopic diseases and autoimmune Th1-mediated diseases such as T1DM are high worldwide (7). However, our study failed to demonstrate a positive relationship between T1DM and the presence of both, self-reported symptoms of IgE-mediated allergies and increased total IgE levels or sensitivity to aeroallergens. This

might be caused by the low number of cases in our study. The results of previous questionnaire surveys (9, 10, 30–38) that investigated the association between T1DM and the presence of allergies are very controversial. In 2012, for example, Fsadni et al. compared the reported country incidence of T1DM in Malta with the prevalence of atopic disease. The incidence of T1DM was found to have a positive correlation with both wheezing and atopic eczema, whereas there was no correlation between the incidence of T1DM and

Table 5. Results of a variance analysis of the multiplex cytokine analysis

|                                            | TNF $\alpha$ |          |          | IL-2         |            |          | IL-6     |              |            | IL-10    |          |              |
|--------------------------------------------|--------------|----------|----------|--------------|------------|----------|----------|--------------|------------|----------|----------|--------------|
|                                            | Difference   | Lower CI | Upper CI | p Value      | Difference | Lower CI | Upper CI | p Value      | Difference | Lower CI | Upper CI | p Value      |
| T1DM : no T1DM                             | 5.62         | 4.08     | 7.15     | <b>0.000</b> | -0.40      | -1.28    | 0.48     | NS           | 1.39       | -1.49    | 4.28     | NS           |
| Allergy* : no allergy                      | 0.69         | -0.98    | 2.36     | NS           | 1.27       | 0.31     | 2.22     | <b>0.009</b> | 4.70       | 1.56     | 7.84     | <b>0.003</b> |
| T1DM and allergy* : no T1DM and no allergy | 6.85         | 3.71     | 9.98     | <b>0.000</b> | 0.03       | -1.77    | 1.83     | NS           | 6.43       | 0.53     | 12.32    | <b>0.027</b> |
| T1DM and allergy* :                        | 1.87         | -1.62    | 5.36     | NS           | -0.02      | -2.03    | 1.98     | NS           | 6.39       | -0.18    | 12.96    | NS           |
| T1DM and no allergy                        | 6.91         | 3.10     | 10.73    | <b>0.000</b> | -2.14      | -4.33    | 0.05     | NS           | 2.71       | -4.47    | 9.89     | NS           |
| T1DM and allergy* : no T1DM and allergy    | 4.98         | 2.58     | 7.38     | <b>0.000</b> | 0.62       | -1.32    | 1.44     | NS           | 0.04       | -4.48    | 4.55     | NS           |
| T1DM and no allergy* :                     | -0.07        | -2.93    | 2.79     | NS           | 2.17       | 0.53     | 3.81     | <b>0.004</b> | 3.72       | -1.66    | 9.09     | NS           |
| No T1DM and allergy* :                     | -5.05        | -8.29    | -1.81    | <b>0.000</b> | 2.11       | 0.25     | 3.97     | <b>0.019</b> | 3.68       | -2.42    | 9.78     | NS           |
| No T1DM and no allergy                     |              |          |          |              |            |          |          |              |            |          |          |              |
| No T1DM and allergy* :                     |              |          |          |              |            |          |          |              |            |          |          |              |
| No T1DM and no allergy                     |              |          |          |              |            |          |          |              |            |          |          |              |

CI, confidence interval; IL, interleukin; NS, not significant; TNF $\alpha$ , tumor necrosis factor alpha

Values &lt; 0.05 were considered as significant and printed in bold.

\*At least one of the four aspects allergic asthma, allergic rhinitis, allergic urticaria, or allergic shock is positive.

the prevalence of rhino-conjunctivitis (31). Another retrospective data analysis from Finland showed that asthma was 1.5 times more common in children with T1DM than in children without T1DM, although the difference was not significant (33). Sheikh et al. analyzed an adult population and was able to show a significant positive association between the history of physician-diagnosed allergic disorders and having a physician-diagnosed Th1-mediated autoimmune condition, such as T1DM, a thyroid or rheumatoid disorder (36). Another study of similar design showed the same association (10). These observations are in agreement with the results of our study which show that T1DM is associated with a higher risk of a self-reported presence of IgE-mediated allergies. In contrast, there are several studies that showed a negative association between T1DM and IgE mediated allergies (9, 30, 32, 34, 35). The controversial findings of previous studies could be explained by not only using different questionnaire items, but also by methodological problems, especially in regards to the identification of atopic children. To avoid this problem we used objective laboratory investigations such as IgE levels in addition to the patients' or parents' reports in the questionnaire. However, we failed to demonstrate a positive relationship between T1DM and the presence of both, self-reported symptoms of IgE-mediated allergies and increased total IgE levels or sensitivity to aeroallergens. One reason might be the low number of cases. There are a few previous studies that investigated the association between T1DM and IgE-mediated allergies by using objective parameters, too (5, 6, 12, 39). In terms of serum IgE levels Cakir et al. and Gazit et al. did not find a significant difference between children with T1DM and healthy controls (6, 12). This is consistent with our results. Using another objective parameter for the presence of IgE-mediated allergies, namely skin-prick tests, Cakir et al. found a higher prevalence of positive skin-prick tests in the healthy control group than in the group of children with T1DM ( $p < 0.05$ ) (6). However, Caffarelli et al. could not demonstrate an association between skin-prick test results and T1DM (5), likewise Tosca et al. (39). The initially planned skin-prick tests in our study were not approved by the Ethical Committee. But we used the existing serum samples of the children to measure cytokine levels with a multiplex cytokine analysis. There are only a few previous studies that investigated cytokine profiles in patients with T1DM and allergic diseases and these showed very controversial results. Rachmiel et al., for example, investigated in 2011 the IL-12 and IL-18 secretion pattern in these two groups, knowing that the two cytokines play an important role in the regulation of both Th1- and Th2-mediated diseases (40). They found that patients with both T1DM and asthma had higher serum levels of

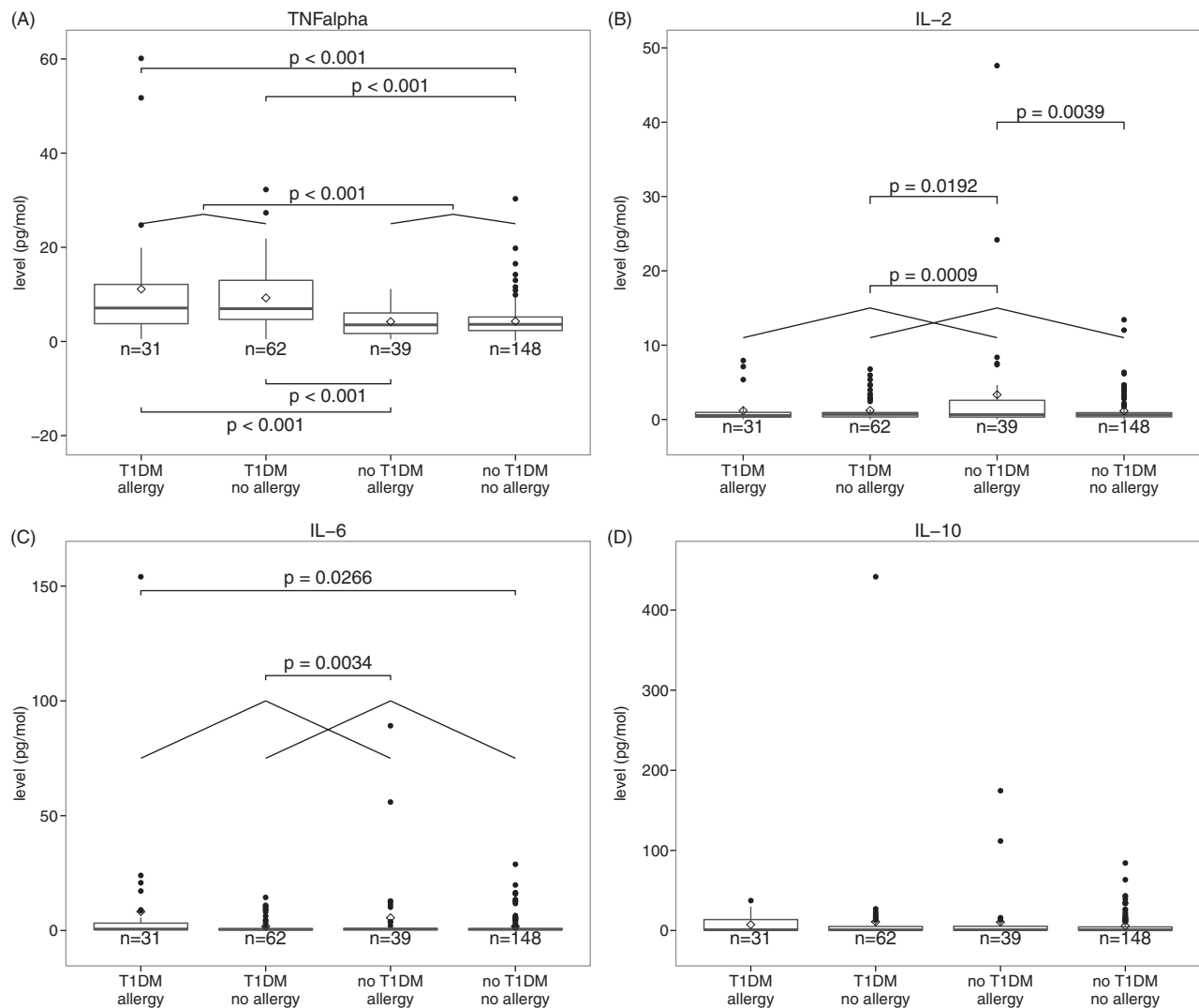


**Fig. 2.** Results of a variance analysis of the multiplex cytokine analysis. In the first row levels of tumor necrosis factor alpha (TNF $\alpha$ ) (A) and interleukin-2 (IL-2) (B) representing Th1 cell cytokines and in the second row levels of IL-6 (C) and IL-10 (D) representing Th2 cell cytokines, respectively in four groups referring to the two items presence of type 1 diabetes mellitus (T1DM) and presence of a positive personal history for allergies in general are depicted. Children with T1DM had significant higher levels of TNF $\alpha$  than children without T1DM, regardless of the presence of a personal history for allergies [(A)  $p < 0.001$ , respectively]. Within the two groups of children with T1DM there was no statistically significant difference in regards to the mean TNF $\alpha$  values, as well as within the two groups of children without T1DM (A). Comparing the two groups of children with positive personal histories for allergies with the two groups of children without allergies there was no statistical difference in regards to the mean TNF $\alpha$  values, either. Children without T1DM, but the presence of a personal history for allergies had significant higher levels of IL-2 than children without allergies, regardless of the presence of T1DM [(B)  $p_1 = 0.0039$ ,  $p_2 = 0.0192$ ]. Comparing the two groups of children with the presence of a personal history for allergies (T1DM, allergy and no T1DM, allergy) with the other two groups (no allergies), levels of IL-2 were significantly higher in the group of children with a positive personal history for allergies [(B)  $p = 0.009$ ]. Comparing these groups analogously in C, levels of IL-6 were significantly higher in the group of children with a positive personal history for allergies [(C)  $p = 0.0034$ ]. Furthermore, children with T1DM and a positive personal history for allergies had significant higher levels of IL-6 than children without these both diseases [(C)  $p = 0.0266$ ]. Between the other groups we did not find significant differences in mean levels of (B) IL-2 and (C) IL-6. Mean values of IL-10 (D) did not differ significantly between any of the groups.

IL-12 and IL-18 than controls (41). Cytokines play an important role in inflammatory processes and therefore also in inducing beta-cell damage in patients with T1DM (15). Furthermore it is supposed that Th2-cytokines, such as IL-5 and IL-4 are involved in the local infiltration and activation of eosinophils, whereas IL-10 and IL-13, also representatives of Th2-cytokines, seem to play an important role in inducing airway

hyperreactivity and allergic inflammation (24). In our study we investigated TNF $\alpha$  and IL-2 levels as representatives of Th1 cytokines and IL-6 and IL-10 levels as representatives of Th2-cytokines. We found that children with T1DM had higher TNF $\alpha$  levels than healthy controls. This result is in agreement with the known fact, that inflammatory activity and accordingly endothelial dysfunction are increased in type 1 diabetes

(42). Higher levels of TNF $\alpha$  levels in every stage of the T1DM indicate the persistence of activation of a systemic inflammatory response (16). TNF $\alpha$  is suggested to mediate damage to microvascular and macrovascular tissues, to alter insulin secretion and glucose homeostasis (43, 44). However, in previous studies results of measuring TNF $\alpha$  levels are reported to be increased (15, 19) in patients with diabetes, decreased (20), or not different (21, 22). The IL-2 system is known to be associated with autoimmune processes and therefore with T1DM, too. Results of measuring IL-2 in patients with T1DM have also been very controversial; IL-2 levels were found to be increased, decreased, or similar to controls (45–47). In our study children without T1DM, but the presence of a personal history for allergies had significant higher levels of IL-2 than children without allergies, regardless of the presence of T1DM. This is contrary to the assumption that the autoimmune process in developing T1DM is dominated by the Th1 subfraction. Comparing the two groups of children with the presence of personal history for allergies with the other two groups (no allergies) levels of IL-6 were significantly higher in the group of children with a positive personal history for allergies. This would support the Th1/Th2-hypothesis and goes along with the findings of Wong et al., who found higher levels of IL-6 in allergic patients, too (24). However, these findings are partially controversial to previous findings on IL-6 levels in patients with T1DM. Targher et al., for example, found elevated levels of IL-6 in this specific group of patients (17), whereas Geerlings et al. found decreased (20) and Kulseng et al. found similar levels compared with healthy control subjects (18). There is a need for further population-based epidemiological studies. Moreover there are newly discovered subsets of Th17 cells that differentiate along a pathway that is totally independent of Th1 and Th2 cells (48). This complexity underlines our hypothesis that the Th1/Th2 paradigm may be an oversimplification. Consistent with the results of our study there is the assumption of some study groups that there might be common factors influencing the development of T1DM and IgE-mediated allergies. Knowing that both diseases have a multifactorial pathogenesis that is influenced by genetic, environmental, and immunological factors, there are a few studies that tried to investigate the association between T1DM and IgE-mediated allergies by considering these factors. Wahlberg et al. found in 2011 that the occurrence of wheezing during the first year of life was strongly associated with the risk of beta-cell autoimmunity at the age of 2.5 yr. It is well-known that wheezing in the first year of life is associated with later development of asthma. Therefore, the findings suggest that there might be common factors influencing the development of both T1DM and allergies (49). Looking

at sociodemographic parameters as another important environmental factor Karavanaki et al. could not observe a social class differentiation in the frequency of allergies (13). A Norwegian study group failed to explain their previous finding of an inverse association between childhood eczema and subsequent risk of T1DM by established diabetes susceptibility genes (38).

To interpret our findings correctly, some characteristics and limitations have to be discussed as follows; first the number of cases ( $n = 282$ ) is quite low. Case and control data was collected during the same period of time by using standardized methods and identical procedures in both groups. Therefore we think that data bias is unlikely. Using a standardized and validated questionnaire, as well as laboratory investigations as objective parameters, is also a strength of our study. However, recall and disease-dependent biases might create appreciable problems in our study. Parents of children with chronic diseases such as T1DM who are seen by a physician on a regular basis are said to be more sensitive in reflecting symptoms of their children than parents of healthy children. The results of our case control study show that T1DM may be associated with a higher risk of self-reported presence of IgE-mediated allergies. Therefore the traditional Th1/Th2 paradigm may be an oversimplification. Both diseases have a complex multifactorial and multigenetic pathogenesis. We are sure that there is a genetic influence on the discrepancies shown, too. However, our case–control-study was not laid out to investigate possible genetic confounders because the number of cases is way too small to perform a genome wide association study or candidate gene approach. Therefore, further population based epidemiological studies involving confounders like environmental, genetic, and immunological factors are needed. Understanding the complexity of the formation mechanism of a possible association between both diseases might be relevant in the future, especially in terms of prevention.

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