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ATP4A autoimmunity and *Helicobacter pylori* infection in children with type 1 diabetes

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Summary

Persistent presence of ATP4A autoantibodies (ATP4AA) directed towards parietal cells is typical for atrophic body gastritis (ABG), an autoimmune disease associated with type 1 diabetes. We assessed whether *Helicobacter pylori* (Hp) infection might be associated with positivity for ATP4AA in children with type 1 diabetes. Sera were collected from 70 (38♀) type 1 diabetes children [aged 13·2 ± 4·5 years, age at diagnosis 8·8 ± 4·3 years, diabetes duration 4·5 ± 3·8 years, mean HbA1c 7·8 ± 1·6% (62 ± 17·5 mmol/mol)] seen at the regional diabetes clinic in Katowice, Poland. Patients were tested concurrently for Hp infection by means of a 13C urea breath test. ATP4AA were measured using a novel radioimmunoprecipitation assay developed at the Barbara Davies Center for Childhood Diabetes, University of Colorado. ATP4AA were present in 21 [30%, 95% confidence interval (CI) = 19–41%] and Hp infection was detected in 23 (33%, 95% CI = 22–44%) children. There was no statistically significant association between ATP4AA presence and Hp status. ATP4AA presence was not associated with current age, age at type 1 diabetes diagnosis, diabetes duration or current HbA1c. ATP4AA were more prevalent in females [42% (26–58%)] than males [16% (3–28%)], $P = 0{\cdot}016$. ATP4A are found in nearly one-third of children with type 1 diabetes and more common among females. In this cross-sectional analysis, Hp infection was not associated with autoimmunity against parietal cells.

Keywords: autoantibodies, autoimmune gastritis, *Helicobacter pylori*, parietal cell, type 1 diabetes

Introduction

The prevalence of *Helicobacter pylori* (Hp) infection in the adult population of European countries ranges from 10 to 30%, but approaches 70% in the developing regions of the world [1]. Hp is an aetiological factor for chronic gastritis, duodenitis, peptic ulcer and a chronic infection that may lead to mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer [2–4]. Moreover, some studies report that Hp may trigger an autoimmune response against the H+/K+ ATPase and be involved in the pathogenesis of autoimmune atrophic body gastritis (ABG) [5–7]. Present gastroenterological guidelines for the paediatric population suggest screening patients with gastric atrophy for Hp infection [8]. None the less, it is noteworthy that a precise relationship between Hp and infection and ABG has not yet been confirmed [9–11].

ABG is found in 0·15-1% of the general population, and the prevalence rises with age. It is more common in patients with other autoimmune diseases, e.g. type 1 diabetes [5,12]. Thus far the determinants of gastric autoimmunity among type 1 diabetes individuals have not been firmly established. Limited data exist concerning the presence of autoantibodies targeting parietal cells (PCA) in children and adolescents with type 1 diabetes, especially with regard to the possibility of concomitant Hp infection [5,10,13]. The prevalence of PCA has been reported to be between 9 and 21% in adults and 4 and 15% in children with type 1 diabetes [5,14]. Most of these studies employed enzyme-linked immunosorbent or indirect immunofluorescence to detect the PCAs that are reactive to both ATPase subunits (4A and 4B) of the gastric proton pump. However, published evidence indicates that the major antigen in ABG is the gastric H+/K+ ATPase 4A subunit (ATP4A) [12,15]. Specific

ATP4A autoantibodies (ATP4AA) emerge early in life (5–10 years) and persist over time. The question arises as to whether their assessment would provide new insights into the association between type 1 diabetes and ABG and be a more accurate biomarker to evaluate early manifestation of ABG.

We investigated whether the prevalence of gastric autoimmunity, determined by measurement of ATP4AA, was related to the Hp infection status in children and adolescents with type 1 diabetes.

Materials and methods

Patients

Seventy individuals (38 females, 32 males), mean age 13·2 ± 4·5 years (range 4–21 years), were recruited in 2012–13 from the regional university paediatric diabetes clinic in Katowice, Poland. The mean age at type 1 diabetes diagnosis was 8·8 ± 4·3 years (range 1–18 years) and disease duration was 4·5 ± 3·8 years (range 0–16 years). The mean HbA1c at the time of the study was 7·8 ± 1·6% (62 ± 17·5 mmol/mol). The inclusion criteria included confirmed type 1 diabetes and co-operation allowing administration of a urea breath test (UBT). Exclusion criteria included other types of diabetes, other autoimmune or gastrointestinal diseases and a history of proton pump inhibitor or antibiotic therapy up to 4 weeks prior to examination.

This study was approved by the institutional ethical committee of the Medical University of Silesia, Katowice, Poland. A written informed consent was obtained from all the parents and/or patients (depending on their age).

Methods

Serum samples for ATP4AA assessment were acquired from all the study participants and ATP4AA were measured by a novel radioimmunoprecipitation assay (RIA) developed and performed at the Barbara Davis Center for Childhood Diabetes, at the University of Colorado School of Medicine (Aurora, CO, USA) [12]. Radioimmunoprecipitation assays were carried out using an ATP4A derivative predicted to lie on the cytosolic face of the membrane as the antigen probe cloned into vector pcDNA3·1 (Invitrogen, Carlsbad, CA, USA). Briefly, we generated a ^{35}S-labelled protein probe with a coupled *in-vitro* transcription/translation system (Promega, Madison, WI, USA), purified by gel filtration. Human sera samples were incubated with 20 000 counts per minute (cpm)/EA of the radiolabelled antigen O/N at 4 degrees and immune complexes captured with protein A Sepharose, transferred to filter plates and sequentially washed with phosphate-buffered saline (PBS) containing 0·1% Tween 20 and 1·0% bovine serum albumin (BSA). Bound radioactivity was assessed by scintillation counting. The assays were performed with 16 matched (negative) control samples and a pool of human sera with high-titre ATP4AA (positive control). The immunoprecipitation index was calculated according to the following equation: (mean of the sample cpm – mean of the negative controls)/(mean of the positive control – mean of the negative controls). The cut-off for the index was determined to be 0·02.

Glycated haemoglobin A1c (HbA1c) of the patients was determined simultaneously. The HbA1c measurements were performed in one laboratory using a Diabetes Control and Complications Trial (DCCT) reference method with a typical normal range of < 6% (< 42 mmol/mol).

At the time of serum collection, patients were tested for the presence of Hp infection while fasting using a UBC with ^{13}C isotope-labelled urea. Breath samples, at baseline and 30 min after ingestion of 75 mg of ^{13}C isotope-labelled urea, were collected in 650 ml aluminized bags with one-way valves. An infrared spectrophotometer (IRIS; Wagner GMBH, Silkerode, Germany) was used to measure the $^{12}CO_2/^{13}CO_2$ ratio. The results of the test were considered positive if ^{13}C concentration [delta over baseline, DOB (o/oo)] rose in the exhaled air by more than 4·0‰. UBC as a non-invasive method has been extensively validated in children, and allows testing for Hp before and after Hp treatment [8].

Statistical analysis

The R software (http://www.bioconductor.org) was employed for statistical analyses. Outlying values were detected using Tukey's criterion. Distribution normality was checked with the Lilliefors test and variance homogeneity hypothesis was tested with *F* or Bartlett statistics. For comparative analysis we used analysis of variance (ANOVA) and Student's *t*-test (normally distributed variables); otherwise, the non-parametric ANOVA Kruskal–Wallis and Mann–Whitney *U*-test were used. Depending on the distribution, Pearson's or Spearman's correlation coefficients were used to estimate the associations between two continuous variables. For discrete variables, the χ^2 or likelihood-ratio G-test was performed. Results were considered as significant at $P < 0{\cdot}05$.

Results

ATP4AA were found in 21 patients [30%, 95% confidence interval (CI) = 19–42%]. The UBC result indicated Hp infection in 23 cases (33%, 95% CI = 22–45%). The UBC was positive in eight ATP4AA-positive patients (38%, 95% CI = 18–62%) and in 15 ATP4AA-negative patients (31%, 95% CI = 18–45%; $P = 0{\cdot}32$) (Fig. 1). The observed discrepancy in the DOB30 values [5·0 (0·3–4·1) *versus* 9·5 (0·4–16·2); $P = 0{\cdot}17$], also did not reach significance. There was no correlation between DOB30 and ATP4AA levels (Spearman's $r = 0{\cdot}06$, $P = 0{\cdot}60$).

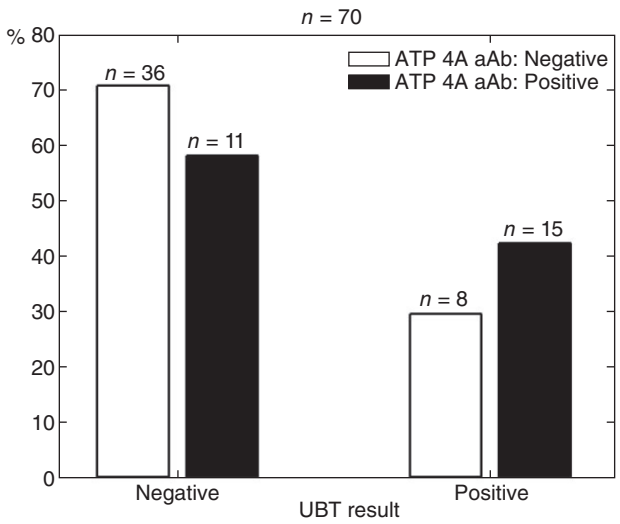

Fig. 1. ATP4A autoimmunity relative to urea breath test (UBT) test results. The 8% difference between patients with *Helicobacter pylori* infection did not reach statistical significance ($P = 0{\cdot}32$). ATP 4A aAb = autoantibodies towards the 4A subunit of the gastric ATPase.

ATP4AA occurrence was independent of age ($P = 0{\cdot}74$). We additionally analysed ATP4AA frequency in two age subgroups (above and below/or equal to 10 years), but also found no significant difference ($P = 0{\cdot}68$). Age at type 1 diabetes diagnosis, disease duration or HbA1c were not associated with the presence of ATP4A ($P = 0{\cdot}68$, $P = 0{\cdot}22$ and $P = 0{\cdot}54$, respectively). Significantly more females were ATP4AA-positive than males (76%, 95% CI = 53–92% *versus* 24%, 95% CI = 8–47%; $P = 0{\cdot}016$). There was no relationship between ATP4AA and the presence of such symptoms as abdominal pain, halitosis, nausea and loss of appetite.

The UBC results were independent of gender and age ($P = 0{\cdot}79$ and $P = 0{\cdot}62$, respectively). Furthermore, diabetes duration ($P = 0{\cdot}45$) and HbA1c ($P = 0{\cdot}22$) showed no relationship with the presence of Hp infection in type 1 diabetes patients. No significant association was found between Hp status and the assessed, potential symptoms of Hp infection (abdominal pain, halitosis, nausea and loss of appetite).

Discussion

We found a higher than expected (30%) prevalence of ATP4AA among young patients with type 1 diabetes. Previous reports have estimated that 4–21% of the patients were PCA-positive [5,13,14,16]. Even in adults the former estimates were lower (9–25%) [5,14,17] than those observed in our study population. This difference might indicate that assessing ATP4AA towards the primary antigen of the proton pump (ATP4A) [12,15] may enhance sensitivity. Additionally, typical PCA seem to develop later in life (second or third decade). It is possible that gastric autoimmunity in our patient cohort was discovered earlier using the ATP4AA than would be detected via the formerly used standard methods using both subunits of the gastric proton pump or the intrinsic factor as antigen [5,10,13]. However, the clinical significance of ATP4AA needs further investigation.

It should be emphasized that our patient population included only subjects with no other concomitant autoimmune disorders, which was not necessarily the case in previous reports. While unlikely, our more homogeneous group of individuals may display higher ATP4AA frequencies than a broad range of type 1 diabetes subjects. Published reports indicated a higher incidence of gastric autoimmunity in individuals with autoimmune thyroiditis and revealed a positive relationship between the presence of PCAs and anti-thyroid peroxidase antibodies among children with type 1 diabetes [5].

The prevalence of Hp infection among this group of individuals was 32%, which is concordant with previous studies in Polish children with type 1 diabetes [18]. Whether Hp prevalence in young type 1 diabetes patients is higher than in the general population remains controversial [19–21]. Although not addressed in the present study, recent studies in Poland report a lower (~16%) infection rate in healthy children [22]. Thus, Hp infection may be associated with type 1 diabetes independently of parietal cell autoimmunity.

The potential role of Hp in triggering the autoimmune process by means of antigenic mimicry towards the gastric proton pump has been suggested previously [5–7,9–11,14,23]. None the less, a positive correlation of PCA and Hp infection was observed in only a few studies [9–11], which was not subsequently confirmed [7,14]. Our results showed that ATP4AA positivity was not related significantly to Hp infection, suggesting that Hp infection may lead to ABG through an alternate mechanism than autoimmunity, despite the molecular similarity of Hp and the gastric proton pump antigens. An earlier study suggested that the relationship may be based on a shared genetic predisposition. Some Hp genes have been found to function in the transcriptional repression of ATP4A and consequently lead to lower activity of gastric H+/K+ ATPase [4,24].

We confirmed that ATP4AA are approximately three times more frequent in females, compared to males, as has been documented earlier, although the relationship between gender and PCA presence remains controversial [5,7,12,14,16,17].

Moreover, we found no impact of age, age at type 1 diabetes diagnosis, disease duration or HbA1c on ATP4AA autoimmunity. A former study monitoring ATP4A antibodies among newly diagnosed type 1 diabetes individuals however, showed, their increasing prevalence with age at diabetes diagnosis [12]. This discrepancy may be explained partially by the smaller study cohort, and thus a tighter range of age of disease onset in our investigation. Previous studies have demonstrated that ATP4AA titres persist, if not

increase, over the course of type 1 diabetes [12]. The results of other studies concerning PCA prevalence as a factor of age of type 1 diabetes onset are also inconsistent [5,13,14,16] with our study supporting the lack of an association between diabetes duration and PCA presence [5,13,14,16].

With respect to Hp, we found no association of the UBC measures with any of the parameters examined. Although epidemiological studies suggest that the prevalence of Hp infection increases with age [22], research conducted in type 1 diabetes subjects does not support this finding [18,20,25]. The negative association of Hp with HbA1c has also been documented by other investigators [19,20,25]. Nevertheless, there are confounding data reported in the literature implying a positive association of Hp with HbA1c [18]. The metabolic control of the patient cohorts in the different studies varied substantially, and the acceptable mean HbA1c levels of our patients is notable. The influence of type 1 diabetes duration on Hp infection has been investigated by only a few other researchers; our findings validate those reported previously [18,20,25].

In summary, almost one-third of type 1 diabetes individuals express ATP4AA. The prevalence of ATP4AA shows a clear female gender bias. The autoimmune process in young patients with type 1 diabetes does not appear to be related to Hp infection as measured by PCA, which suggests that the relationship between Hp and ABG could be based on an alternative pathological mechanism. Further studies are needed to explain the clinical relevance of APT4AA, clarify their role in autoimmune gastritis and determine if patients should be screened for ATP4AA presence.

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Disclosure

The authors have no conflicts of interest to disclose.

Author contributions

A. C. conceptualized and designed the study, designed the data collection instruments, drafted the initial manuscript, and approved the final manuscript as submitted. J. W. carried out the laboratory analysis and interpreted the laboratory results. J. P. carried out the initial analyses. K. B.-D. co-ordinated and supervised the data collection. J. K. designed the data collection instruments. M. R. helped to design the study. A. C., J. W., K. B.-D., J. K., J. P. and M. R. critically reviewed the manuscript and approved the final manuscript as submitted.

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