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ORIGINAL ARTICLE

Exocrine pancreas function decreases during the progression of the beta-cell damaging process in young prediabetic children

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Objective: The function of the exocrine pancreas is decreased in patients with type 1 diabetes but it is not known when this defect develops. The current study set out to determine whether the reduced exocrine function becomes manifest after the initiation of islet autoimmunity.

Methods: The study was nested in the prospective Type 1 Diabetes Prediction and Prevention study where children with human leukocyte antigen (HLA)-conferred susceptibility are observed from birth. Elastase-1 levels were analyzed from stool samples collected at the time of seroconversion to islet autoantibody positivity and at diagnosis of type 1 diabetes, as well as from samples taken from matched control children of similar age.

Results: Elastase levels were lower in case children at the time of the diagnosis of diabetes when compared to the control children. However, elastase concentrations did not differ between cases and controls at the time when autoantibodies appeared.

Conclusion: The results suggest that the defect in the exocrine function develops after the appearance of islet autoantibodies. Further studies are needed to assess whether reduced elastase levels predict rapid progression of islet autoimmunity to clinical disease.

KEYWORDS

autoantibodies, pancreatic elastase, prediabetic state, seroconversion, type 1 diabetes mellitus

1 | INTRODUCTION

Previous studies have suggested that the function of the exocrine pancreas is impaired in 43% to 80% of patients with type 1 diabetes.[1–3] However, the underlying mechanisms are poorly understood, and several hypotheses have been put forward, including an autoimmune process against exocrine pancreas, a decreased trophic effect of insulin on the exocrine pancreas, microangiopathy, atherosclerosis and diabetic neuropathy.[4] It has also been hypothesized that pancreatic insufficiency could be related to a specific subtype of diabetes in which the whole organ is affected (type 3c diabetes).[5,6]

Recent studies have provided new insights into the disease process affecting pancreas tissue in type 1 diabetes. One of the key findings is that the size of the whole organ is clearly reduced, and this decrease can be seen already within months after the diagnosis of type 1 diabetes.[7] Another study carried out in the nPOD (Network for Pancreatic Organ Donors with Diabetes) collection showed that the weight of the pancreas was reduced not only in patients with type 1 diabetes but also in prediabetic individuals who tested positive for islet autoantibodies.[8] In addition, studies in the nPOD collection have shown that exocrine tissue is affected by inflammation.[9–11]

The present study evaluates whether elastase concentrations in stools, as one of the most widely used biomarker of the function of exocrine pancreas, is reduced in children with newly diagnosed type 1 diabetes, and whether a possible reduction can be observed already at the early preclinical stage of the disease, close to the time when the first autoantibodies appear.

2 | METHODS

2.1 | Study design

The study cohort included 81 case children who had either newly diagnosed type 1 diabetes (n = 28) or who were non-diabetic but positive for multiple islet autoantibodies (n = 53). The autoantibody positive children were recruited in the Type 1 Diabetes Prediction and Prevention (DIPP) birth cohort study, which observes children with increased human leukocyte antigen (HLA)-conferred susceptibility to type 1 diabetes from birth in 3 regions in Finland. Serum and stool samples have been collected regularly during the follow-up and islet cell antibodies (ICA), insulin autoantibodies (IAA), glutamic acid decarboxylase antibodies (GADA) and insulinoma-associated protein 2 antibodies (IA-2A) have been analyzed from all follow-up sera as described previously.[12]

Study subjects were selected from the DIPP cohort on the basis of availability of a stool sample taken at the time when the first autoantibodies were detected and/or at the time of diagnosis of type 1 diabetes. The participants were born between 1996 and 2008, 63% were boys and the median age at autoantibody seroconversion was 1.3 years (range 0.4-4.1 years). All non-diabetic case children (n = 53) were positive for at least 2 of the tested autoantibodies and 41 (77%) had progressed to clinical type 1 diabetes. Their median age at the time of diagnosis was 4.9 years (range 1.6-13.0 years). The DIPP children carried HLA risk alleles for type 1 diabetes (HLA-DQB1*02/*0302, DQB1*0302/x [x ≠ *02, *0301 or *0602] or DQB1*02/y-DQA1*05/z [y ≠ *0301, *0302,*0602,*0603; z ≠ *0201] genotypes). A series of children with newly diagnosed type 1 diabetes (n = 19) was in addition available from Tampere University Hospital in Finland. These children were born between 1990 and 2003, 68% were boys and the median age at diagnosis was 9.2 years (range 1.4-14.5 years). One non-diabetic and islet autoantibody negative control child was selected from the DIPP cohort for each case child and matched for calendar time of birth (± 6 months), sex, date of sampling (± 2 months), city of residence and HLA-DQ alleles (described above). The demographics of study subjects are summarized in Table S1, Supporting Information.

All study subjects had written parental consent to take part in the study. The Ethics Committees of Oulu, Tampere and Turku University Hospitals in Finland have approved the DIPP study protocol.

2.2 | Measurement of stool elastase concentration

A stool sample was available for elastase analyses from the time of autoantibody seroconversion from 53 case children (samples taken from 11 weeks before to 14 weeks after the first autoantibody positive serum) and from the time of diabetes diagnosis from 28 case children (taken from 9 weeks before to 1 week after diagnosis). Three children had samples from both time-points. In addition, long series of stool samples were available from 3 additional children to evaluate changes in elastase levels over time (mean 11 samples per child). All stool samples were stored at –20°C to –70°C until analyzed.

Pancreatic elastase-1 (PE-1) was measured using a commercial enzyme-linked immunosorbent assay according to the manufacturer's protocol (ScheBo Biotech, Giessen, Germany). Stool specimens were prepared by the E1 Quick-Prep device (ScheBo Biotech, Giessen, Germany) and the sample was diluted 56-fold before analyses. The results were expressed in μg/g of stool, and values less than

200 μg/g were considered as markers of exocrine pancreatic insufficiency and values less than 100 μg/g as markers of severe insufficiency in both case and control children according to the manufacturer's instructions.

2.3 | Statistical methods

Statistical analysis was performed using the Student's t-test for 2 groups and the χ^2 test. Assumptions were that the response variable, elastase concentration, is normally distributed and variances in both groups are similar. Log transformation was performed to get variable normally distributed. Levene's test for equality of variances and t-test was performed for log transformed data using R 3.2.2 (www.r-project.org). χ^2 test was performed by SPSS (IBM SPSS Statistics for Windows; version 23.0. Armonk, NY, USA). For all statistical tests, a significance level of .05 was applied (2-sided, $P < .05$).

3 | RESULTS

Elastase levels did not differ between the 53 case and 53 control children at the time of islet autoantibody seroconversion (mean 1013 versus 1203 μg/g; median 738 versus 877 μg/g, $P = .149$; Figure 1A). Low elastase levels (< 200 μg/g of stool) were seen in 5 (9.4%) of the case children and in 1 (1.9%) of their controls ($P = .093$).

Elastase levels were significantly lower in stool samples collected from the 28 newly diagnosed type 1 diabetic patients compared to their matched controls (mean 355 versus 828 μg/g; median 230 versus 483 μg/g, $P = .0006$; Figure 1B). Low levels (<200 μg/g of stool) were seen in 11 (39.3%) of the case children and in 2 (7.1%) controls ($P = .004$).

Among the 41 children who progressed to clinical diabetes low elastase levels at autoantibody seroconversion did not explain the
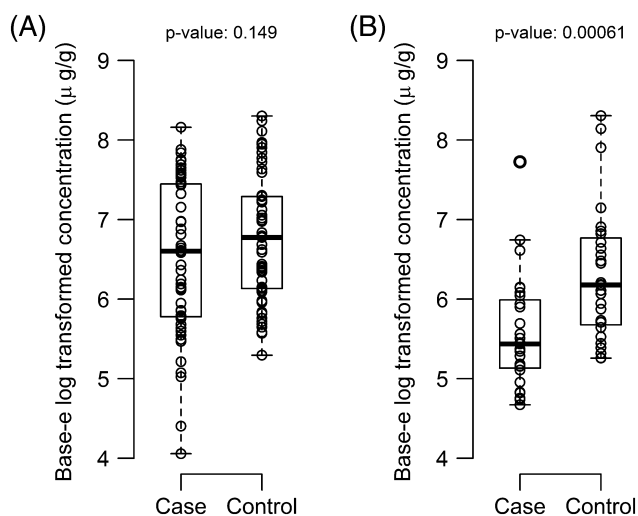

FIGURE 1 Pancreatic elastase-1 concentration levels in stool samples in children with diabetes-associated autoantibodies (A) and in children with type 1 diabetes (B) and in their matched controls. Box plots represent the data after base-e log transformation. Each box plot represents the median (horizontal line), interquartile range and the lowest and highest values (error bars). Values of individual children are shown as circles

speed of progression even though a tendency for faster progression was seen ($P = .1033$ in linear regression analysis). Five of them (12%) had initially low elastase levels (< 200 μg/g), and they progressed to diabetes in 61.3 months (mean) compared to 48.1 months in children with higher elastase levels.

A large set of follow-up stool samples was available from 3 children ranging from the age of 3 to 6 months to the diagnosis of diabetes. A decreasing trend was seen in elastase levels towards diabetes in all of them. In 1 patient, elastase levels decreased gradually showing considerable fluctuation (Figure 2A). In another patient elastase levels rapidly declined soon after autoantibody seroconversion and stayed at low levels until diabetes was diagnosed (Figure 2B). In the third patient, the elastase concentration decreased sharply at 3.5 months of age already before islet autoantibody seroconversion and remained at low levels until clinical diabetes was diagnosed (Figure 2C).

4 | DISCUSSION

This is the first study where the exocrine function of the pancreas has been evaluated during the early preclinical stage of type 1 diabetes by measuring elastase levels in stool samples. The results suggest that while the exocrine function is clearly decreased at the diagnosis of clinical type 1 diabetes this decrease is not present at the time when islet autoantibodies appear. This suggests that in most cases the defect in the function of the exocrine pancreas develops after the initiation of islet autoimmunity. This indicates that this defect is linked to the diabetic disease process per se and is not an inherited character of diabetic patients. The few cases where several longitudinal stool samples were available from different stages of the beta-cell damaging process showed marked individual variation in the decrease of elastase levels over time suggesting that in some children the levels may decrease steeply even within a few months, while in some others this decrease may emerge gradually showing considerable fluctuation. In 1 case, a rapid decrease occurred already some months before the detection of islet autoantibodies. However, since the autoantibodies were measured from sera collected with 3 to 6 months interval, the decrease in elastase occurred probably at the same time when autoantibodies truly appeared. Altogether, these results suggest that repeated measurements of stool elastase levels offer a feasible way to identify a significant part of children whose pancreas function decreases prior or after the diagnosis of type 1 diabetes. As a non-invasive method this could be used as a primary screening tool in young children, and be supplemented by confirmatory assays such as measurement of serum lipase levels.[13] The measurement of elastase concentrations in stools by enzyme-linked immunosorbent assay (EIA) has long been used to evaluate exocrine pancreatic function in clinical practice.[14,15] Elastase is synthesized by the acinar cells along with the other digestive enzymes, and it is not significantly degraded during intestinal transit. Its concentration in human feces is about 5-to 6-fold higher than in pancreatic-duodenal juice. It is also stable in stool samples for up to 1 week at room temperature[13] and is not degraded during a long-term storage at −20°C.[16,17] The good stability during storage at freezer was also suggested by our observation that elastase concentrations did not differ
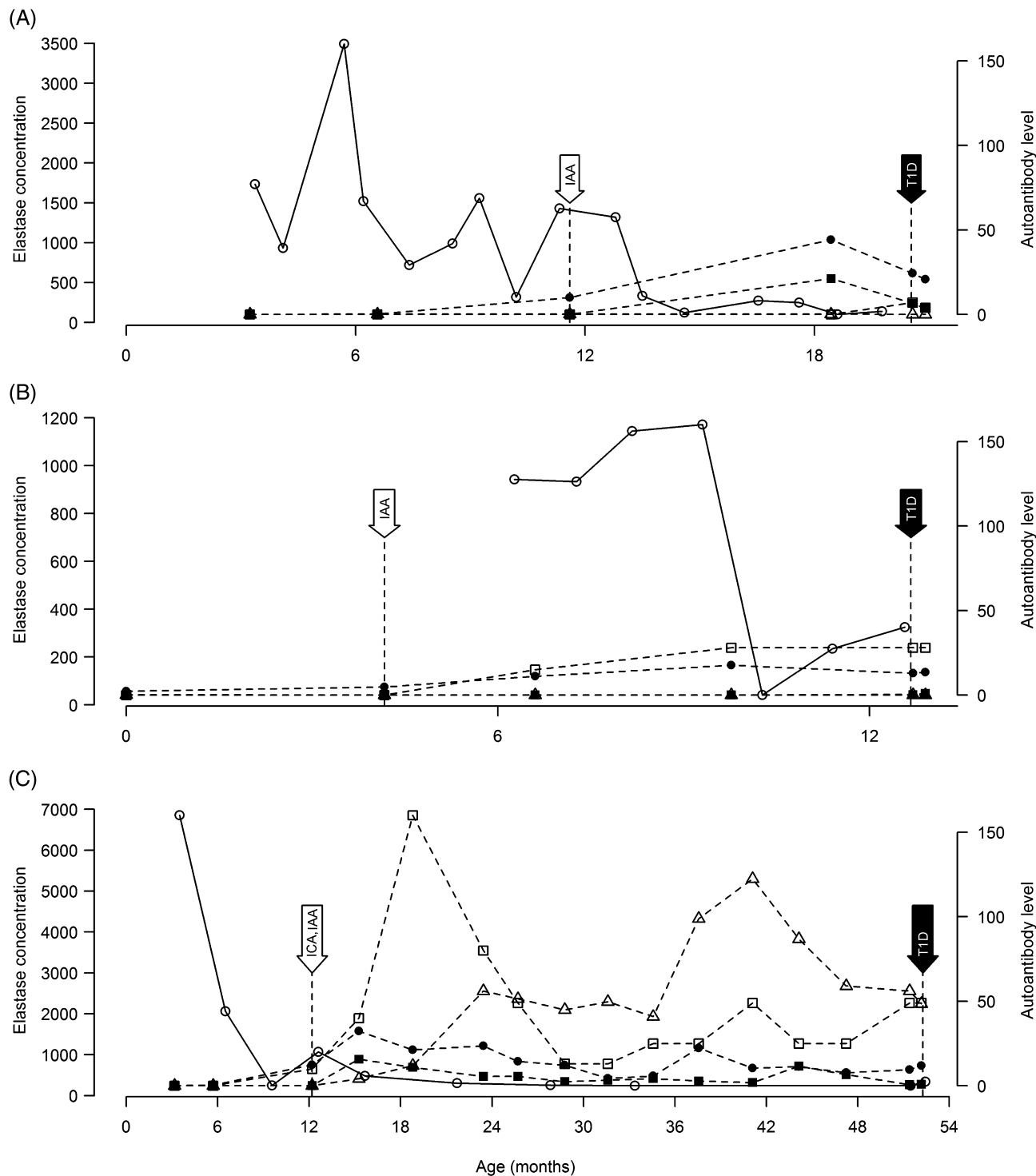
FIGURE 2 Pancreatic elastase-1 concentration in serial stool samples collected from 3 children who were followed from birth and turned positive for islet autoantibodies and progressed to clinical type 1 diabetes (panels A, B and C). The time of the collection of the first islet autoantibody positive serum and the time of the diagnosis of type 1 diabetes are marked by white and black arrows, respectively. —○—, pancreatic elastase-1; --●--, insulin autoantibodies (IAA); --□--, islet cell autoantibodies (ICA); --■--, glutamic acid decarboxylase autoantibodies (GADA); --△--, insulinoma-associated protein 2 antibodies (IA-2A). Cutoffs of autoantibody units are described in Table S1, Supporting Information

between samples with the longest and shortest storage time (median elastase levels 1244 versus 1369 µg/g, P = .8238). This comparison was done using only samples collected from the control children.

Our results are in line with previous observations showing that the exocrine pancreas of type 1 diabetic patients is affected by an inflammatory process and fibrosis[18] and that this process

can start already before type 1 diabetes is diagnosed. Recent studies have also shown that the size of the whole organ is decreased both in patients and in autoantibody-positive prediabetic individuals.[7,8] Altogether, these studies suggest that type 1 diabetes is not only a disease of the beta-cells, but a disease which may affect the whole pancreas. It is unclear whether the

exocrine dysfunction is caused by the same mechanisms as the beta-cell damaging process or if it is secondary to endocrine damage and/or inflammatory changes.

The observed decrease in elastase levels in 40% of the patients with newly diagnosed type 1 diabetes is in line with previous publications.[13] Thus, even if the average elastase levels are lower in patients than in controls only few of them may have clinically significant pancreas insufficiency. In any case, the present study clearly shows that elastase is associated with advanced beta-cell damage in young children. As the study included only young children and the majority of the samples were collected before the age of 3 years, it was not possible to study the occurrence of pancreatic insufficiency in older children.

In conclusion, the results suggest that a defect in exocrine function develops in about 40% of the patients during the progression of islet autoimmunity to clinical type 1 diabetes. This may open possibilities to use stool elastase levels as a biomarker for the progression of the beta-cell damaging process. As the method is simple, non-invasive and technically easy to perform, further studies are indicated to find out whether it could offer a new tool to predict the development of type 1 diabetes in autoantibody-positive individuals.

Conflict of interest

The authors declare no potential conflict of interests.

Author contributions

A.K. researched the data and wrote the manuscript. N.N. selected the study subjects and samples, supervised laboratory analyses and reviewed/edited manuscript, J.L performed statistical analysis and reviewed manuscript, J.T., J.I., R.V., M.K., H.H. contributed to the recruitment of study subjects, collection of samples and reviewed/edited the manuscript. H.H. and M.H. designed the study, contributed to the discussion and reviewed/edited the manuscript. H.H is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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