Penno Megan (Orcid ID: 0000-0002-9617-0826)
Craig Maria (Orcid ID: 0000-0001-6004-576X)
Haynes Aveni (Orcid ID: 0000-0001-9954-5016)
Thomson Rebecca (Orcid ID: 0000-0002-7807-4144)
Couper Jennifer (Orcid ID: 0000-0003-4448-8629)

Full title:

Changes in pancreatic exocrine function in young at-risk children followed to islet autoimmunity and type 1 diabetes in the ENDIA study

Short title:

Pancreatic exocrine function before type 1 diabetes

Authors:

Megan AS Penno PhD[1], Helena Oakey PhD[1], Priya Augustine MBBS[2], Mario Taranto PhD[3], Simon C Barry PhD[1], Peter G Colman MD[4], Maria E Craig PhD[5,6], Elizabeth A Davis PhD [7], Lynne C Giles PhD [8], Mark Harris MD[9,10], Aveni Haynes PhD[7], Kelly McGorm PhD[1], Grant Morahan PhD[11], Claire Morbey MBChB[12], William D Rawlinson PhD[13,14], Richard O Sinnott PhD[15], Georgia Soldatos PhD[16,17], Rebecca L Thomson PhD[1], Peter J Vuillermin PhD[18,19], John M Wentworth PhD[4,20], Leonard C Harrison DSc[20], Jennifer J Couper MD[1,2], on behalf of the ENDIA Study Group[21]

Affiliations:

1. Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA 5005, Australia.
2. Department of Diabetes and Endocrinology, Women's and Children's Hospital, Adelaide, SA 5006, Australia
3. PathWest Laboratories, Fiona Stanley Hospital Network, Murdoch, WA, 6150, Australia
4. Department of Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, VIC 3050, Australia
5. School of Women's and Children's Health, Faculty of Medicine, University of New South Wales, Sydney, NSW 2052, Australia

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6. Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, Sydney, NSW 2145, Australia
7. Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Perth, WA 6009, Australia
8. Robinson Research Institute, School of Public Health, University of Adelaide, Adelaide, SA 5005, Australia
9. The University of Queensland Diamantina Institute, Faculty of Medicine, The University of Queensland, Translational Research Institute, Woolloongabba, QLD 4102, Australia
10. Queensland Children's Hospital, South Brisbane, QLD 4101, Australia
11. Centre for Diabetes Research, Harry Perkins Institute of Medical Research, The University of Western Australia, Perth, WA 6009, Australia
12. Hunter Diabetes Centre, Newcastle, NSW 2291, Australia
13. Virology Research Laboratory, Serology and Virology Division, South Eastern Area Laboratory Services Microbiology, Prince of Wales Hospital, Sydney, NSW 2031, Australia
14. School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW 2052, Australia
15. Melbourne eResearch Group, School of Computing and Information Services, University of Melbourne, Melbourne, VIC 3010, Australia
16. Monash Centre for Health Research and Implementation, School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC 3168, Australia.
17. Diabetes and Vascular Medicine Unit, Monash Health, Melbourne, VIC 3168, Australia
18. Faculty of Health, School of Medicine, Deakin University, Geelong, VIC 3220, Australia
19. Child Health Research Unit, Barwon Health, Geelong, VIC 3220, Australia
20. Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC 3052, Australia.
21. http://www.endia.org.au/the-endia-team

Corresponding author:

Professor Jenny Couper, Women's and Children's Hospital, Adelaide, SA 5006, Australia, Telephone: +61406423880. Fax: +61881617031. Email: jennifer.couper@adelaide.edu.au

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The ENDIA study has been approved by the Women's and Children's Hospital Network Human Research Ethics Committee (HREC) as the lead HREC in South Australia, Queensland, New South Wales, Victoria and regional Australia under the Australian National Mutual Acceptance Scheme (reference number HREC/16/WCHN/066). Conduct in Western Australia has been approved by the Women and Newborn Health Service Ethics Committee (reference number RGS0000002639). The ENDIA study is registered on the Australia New Zealand Clinical Trials Registry (ACTRN1261300794707).

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Author contributions:

JJC conceived the study. JJC, MASP and PA designed the study. PGC, MEC, EAD, MH, AH, KM, CM, WR, GS, PV, JMW, LCH and JJC recruited participants and oversaw collected of biospecimens. PA, MT, PGC and GM acquired the data. JJC, MASP, HO, PA, SCB, ROS and RLT compiled, analysed and interpreted the data. HO and LCG undertook the statistical analysis. MASP, HO and JJC wrote the manuscript. All authors provided critical revision of the manuscript and approved the final version. JC 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.

Conflict of interest:

None declared.

Abstract:

Aim: We aimed to monitor pancreatic exocrine function longitudinally in relation to the development of islet autoimmunity and type 1 diabetes in at-risk children with a first-degree relative with type 1 diabetes, who were followed prospectively in the Environmental Determinants of Islet Autoimmunity (ENDIA) study.

Methods: Fecal elastase-1 (FE-1) concentration was measured longitudinally in 85 ENDIA children from median age 1.0 (IQR 0.7,1.3) years. Twenty-eight of 85 children (progressors) developed persistent islet autoantibodies at median age of 1.5 (IQR 1.1,2.5) years, of whom 11 went on to develop clinical diabetes. The other 57 islet autoantibody-negative children (non-progressors) followed similarly were age and gender-matched with the progressors. An adjusted linear mixed model compared FE-1 concentrations in progressors and non-progressors.

Results: Baseline FE-1 did not differ between progressors and non-progressors, or by HLA DR type or proband status. FE-1 decreased over time in progressors in comparison to non-progressors (Wald statistic 5.46, p=0.02); in some progressors the fall in FE-1 preceded the onset of islet autoimmunity.

Conclusions: Pancreatic exocrine function decreases in the majority of young at-risk children who progress to islet autoimmunity and type 1 diabetes.

Keywords:

children, exocrine pancreas, islet autoimmunity, type 1 diabetes, fecal elastase

Introduction:

The exocrine pancreas is known to be altered in the pathophysiology of type 1 diabetes (T1D). Subclinical exocrine dysfunction was detected in T1D as early as 1943 (1), with numerous confirmations in all age groups (2-5). The exocrine pancreas comprises greater than 95% of the pancreas volume and magnetic resonance imaging shows that the volume decreases stepwise from healthy controls, to first-degree relatives, to first-degree relatives with islet autoimmunity (IA), to individuals with recent-onset T1D and those with longer duration T1D (6, 7). Furthermore, immune cell infiltrates are seen in the exocrine pancreas of organ donors with T1D or IA (8).

Studies of pancreas volume and exocrine function are cross-sectional therefore their temporal relationship with progression to IA and T1D is unknown. Cross-sectionally, young Finnish children with IA had normal exocrine function, as measured by fecal elastase (3), in contrast to reports of smaller pancreatic volume and modestly lower serum trypsinogen levels in older children and adults with multiple islet autoantibodies (4). Given the heterogeneity of progression to T1D, only longitudinal studies can inform as to when measures of exocrine function change in relation to the development of IA and T1D, and whether exocrine function could be used as a predictive biomarker for stratification and selection of subjects into prevention trials.

Fecal elastase is a robust, non-invasive marker of pancreatic exocrine function. The enzyme is stable with minimal degradation during bowel transit over approximately 48 hours. Fecal elastase levels correlate well with the output of pancreatic elastase and do not vary in relation to daily food intake. A recent meta-analysis revealed high sensitivity and specificity of fecal elastase in comparison with fecal fat excretion in children (9).

We aimed to characterise pancreatic exocrine function longitudinally in relation to the development of IA and T1D in at-risk children participating in the Australia-wide prospective

Environmental Determinants of Islet Autoimmunity (ENDIA; ACTRN1261300794707) study (10). ENDIA follows children with a first-degree relative with T1D from the pregnancy. Follow-up is three-monthly until age two, then six-monthly thereafter until the development of T1D. Recruitment of 1500 mother-infant dyads was completed in December 2019. We hypothesised that pancreatic exocrine function would decrease in children after the onset of IA.

Methods:

Participants

Fecal elastase-1 (FE-1) was measured in 247 stool samples from 85 ENDIA children (41:44 male:female; 28 progressors, 57 non-progressors; Table 1) followed from a median age of 1.0 (IQR 0.7,1.3) years for 1.2 (IQR 0.7,2.0) years and with a median of three samples (IQR 2,4). Progressors were the first 28 children who developed persistent IA defined as one or more islet autoantibodies (Ab) to insulin (I), glutamic acid decarboxylase 65,000 Mr isoform (GAD), tyrosine phosphatase-related islet antigen 2 (IA-2) and zinc transporter 8 (ZnT8), measured on at least two consecutive occasions at three-monthly intervals. Of these 28 children, at the last point of follow-up, six had a persistent single islet autoantibody, 11 had multiple islet autoantibodies, and 11 had developed T1D. Of the six with a persistent single islet autoantibody, five had IAb and were HLA DR4X. Screening for T1D was three-monthly by measuring blood glucose after food as defined by the American Diabetes Association. No further visits occurred post-T1D diagnosis. Median age at IA seroconversion was 1.5 (IQR 1.1,2.5) years and T1D diagnosis was at 2.4 (IQR 1.5,2.5) years. Non-progressors were 57 islet autoantibody-negative age and gender-matched children followed at the same time intervals as their matched progressors. No participants were lost to follow-up. Median age of the total ENDIA cohort at the time of the analysis (October 2019) was 2.3 years.

Laboratory assays

FE-1 was measured by ELISA (ScheBo Biotech AG; normal range >200µg/g; interassay CV 10.8%). Islet autoantibodies were measured three-monthly; IAb by radiobinding assay and GADAb, IA-2Ab and ZnT8Ab by the 3-Screen ICA ELISA (RSR Ltd, Cardiff). Positive 3-Screen assays were subjected to individual ELISAs for GADAb, IA-2Ab and ZnT8Ab for confirmation. The assays had 98%, 97%, 100% and 94% specificity, and 28%, 78%, 60% and 72% sensitivity for IAb, GADAb, IA-2Ab and ZnT8Ab respectively, in the 2018 Islet Autoantibody Standardization Program (University of Florida).

Biostatistics

We compared the predicted slopes of regression lines across time for participants in a linear mixed model fitted to determine whether FE-1 changed differentially over time in progressors and non-progressors, in which time was centred by age at seroconversion (in four children IA was detected for the first time at the diagnosis of T1D). Analysis was performed using R (v3.6.3) and packages 'predictmeans' (v1.0.2) and 'lme4' (v1.1.21). The model included the interaction between status (progressor versus non-progressor) and time, and was adjusted for the potential confounders of gender, age at seroconversion and birthweight. Each progressor had up to three age and gender-matched non-progressors that together formed a nest or matched group. The analysis included a random intercept term for nest with 28 levels each representing a matched group within the data set. The inclusion of this term reflects an adjustment for variation in FE-1 levels between the different nests. Log transformation was used to meet model assumptions. A post hoc sensitivity analysis was performed after removing the two points at which FE-1 increased steeply in two non-progressors at the end of the study (Figure 1A).

Results:

Progressors had a decrease in FE-1 over time in comparison to non-progressors (Wald statistic 5.46, p=0.02; unadjusted analysis Wald statistics 5.71, p=0.02), Figure 1B. These findings did not alter significantly after the removal of the two outlying points at which time FE-1

increased steeply to greater than 7500µg/g in two non-progressors (Wald statistic 4.85, p=0.03), Figure 1A. In the six-month windows before and after IA seroconversion, FE-1 increased by 109µg/g from a predicted mean of 1574 (95% CI 1418,1747) to 1683 (1514,1871) µg/g in non-progressors, but decreased by 95µg/g from a predicted mean of 1676 (95% CI 1479,1899) to 1581 (1397,1790) µg/g in progressors. FE-1 decreased in 8/28 progressors between at least two measurements before IA seroconversion. No child had FE-1 concentrations below the lower threshold of 200µg/g for the normal range; concentrations ranged from 229-9150µg/g. Baseline FE-1 (at first data collection point) did not differ according to status (progressor/non-progressor), HLA DR type (DR3,4; DR3,X or 4X; DRX,X), gender, or whether the mother had T1D (42/85 participants).

Discussion:

We report for the first time a decrease in pancreatic exocrine function in young at-risk children who progressed to IA or T1D and in some, from before the time of IA. In contrast, exocrine function continued to rise overall in the non-progressors during the first two years of life, consistent with normal ontogeny (11).

The possibility that children who develop T1D have a smaller pancreas from birth is not supported by our finding that baseline FE-1 in the first year of life was similar in progressors and non-progressors. This must be qualified, however, by the fact that all children were first-degree relatives of individuals with T1D who could have a smaller pancreatic volume even in the absence of IA (6). The decrease in pancreatic volume and exocrine function before the onset of clinical T1D has been attributed to the lack of an insulin-trophic effect on the acinar cells as beta cells are destroyed (12). However, this may not be the complete explanation because we found a decrease in FE-1 before and around the time of seroconversion. While beta cell dysfunction can exist more than 5 years before the onset of type 1 diabetes (13) it has not been demonstrated before the development of IA. The presence of an immune cell infiltrate in the exocrine pancreas (8) suggests that the decrease in pancreatic volume and

exocrine function is likely to be a reflection of pancreas-wide pathology leading to loss of pancreatic beta cells (14).

The limitation of our study is the relatively small number of progressors. Even so, the number is greater than previously reported and the ENDIA children were sampled frequently. Previously, exocrine function was documented in only three children who developed IA (3). Even cross-sectional analyses of pancreas volume and exocrine function is limited in this very young age group at high risk of progression to T1D (2, 3). Progressors included children who had developed both single and multiple autoantibodies as well as children who had progressed to T1D. Cross-sectionally serum trypsinogen was lower in adolescents and young adults with multiple, but not single, islet autoantibodies (4). However, a persistent single islet autoantibody in the first two years of life, especially IAb, confers a considerably higher risk of progression to multiple autoantibodies and T1D than in adolescents and young adults (15).

We conclude that pancreatic exocrine function decreases, within the normal range, at different stages of the progression to IA and T1D in very young at-risk children. Nevertheless, the high variability in FE-1 concentrations, the normal increase in FE-1 during the first years of life, a lack of features distinguishing progressors in whom FE-1 did or did not decrease, and the rapid progression to diabetes in this age group may all limit the role of FE-1 as a biomarker for progression to T1D in early life.

Table 1. Characteristics of the 85 children with longitudinal FE-1 measurements. Islet autoantibody abbreviations: IAb=insulin, GADAb=glutamic acid decarboxylase 65,000 Mr isoform, IA-2Ab=tyrosine phosphatase-related islet antigen 2, ZnT8Ab=zinc transporter 8.

Group (n; male:female)	Status (n)	Median sampling age in years	First appearing islet autoantibody (n)	HLA (n)
Progressors (28; 14:14)	Persistent single IA (6)	2.3	IAb (5) GADAb (1)	DR3,4 (1) DR3,X or 4,X (4) Unknown (1)
	Persistent multiple IA (11)	2.1	IAb + GADAb + ZnT8Ab (1) IAb + GADAb (3) IAb + ZnT8Ab (2) IAb (1) GADAb (3) ZnT8Ab (1)	DR3,4 (4) DR3,X or 4,X (4) DRX,X (2) Unknown (1)
	Progression to type 1 diabetes (11)	1.4	IAb + GADAb + IA-2Ab + ZnT8Ab (1) IAb + GADAb + IA-2Ab (1) IAb + GADAb (2) IAb + IA-2Ab (1) GADAb + IA-2Ab (1) IAb (5)	DR3,4 (5) DR3,X or 4,X (3) DRX,X (1) Unknown (2)
Non-Progressors (57; 27:30)	Islet autoantibody-negative (57)	1.8	-	DR3,4 (11) DR3,X or 4,X (30) DRX,X (14) Unknown (2)

Figure 1. (A) FE-1 concentrations in the 28 progressors and 57 age and gender-matched non-progressors plotted for each participant against time (months) before and after IA seroconversion. Progressors include those progressing to a single islet autoantibody (n=6 as shown by green lines), multiple islet autoantibodies (n=11 blue lines) or T1D (n=11 purple lines, in 4/11 of whom IA was detected for the first time at T1D diagnosis). Each line represents one participant. The starred lines represent the participants who were removed before the post hoc sensitivity analysis. (B) FE-1 concentrations after log transformation in the 28 progressors and 57 age and gender-matched non-progressors. The estimated slope and 95% confidence intervals of the linear model are shown. Progressors include those progressing to a single islet autoantibody (n=6 as shown by green dots), multiple IA (n=11 blue dots) or T1D (n=11 purple dots). FE-1 decreased in progressors in comparison with non-progressors (Wald statistic 5.46, p=0.02).

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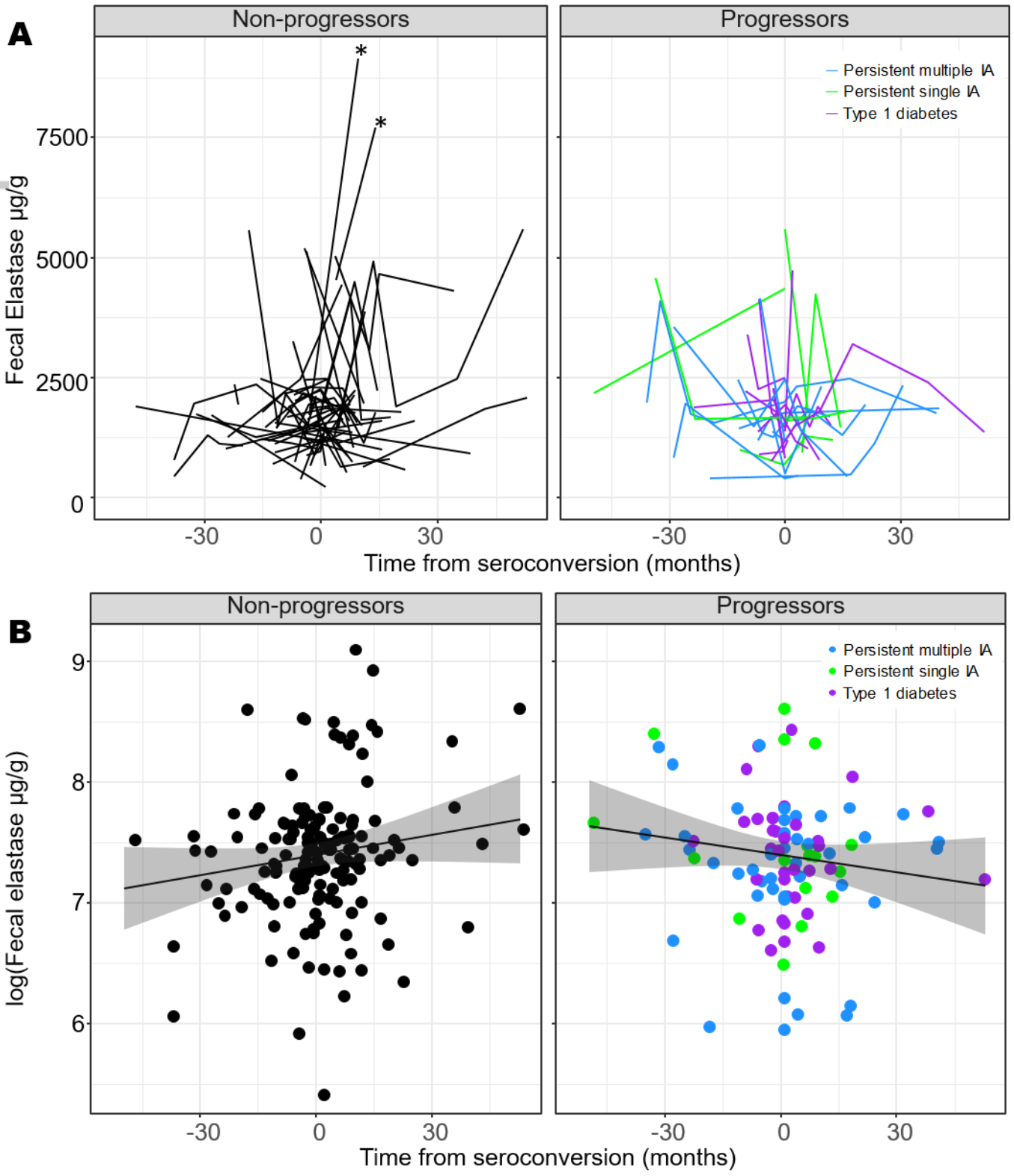

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Author/s:
Penno, MAS; Oakey, H; Augustine, P; Taranto, M; Barry, SC; Colman, PG; Craig, ME; Davis, EA; Giles, LC; Harris, M; Haynes, A; McGorm, K; Morahan, G; Morbey, C; Rawlinson, WD; Sinnott, RO; Soldatos, G; Thomson, RL; Vuillermin, PJ; Wentworth, JM; Harrison, LC; Couper, JJ

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