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Complete List of Authors:	Ronkainen, Jukka; Oulun Yliopisto Laaketieteellisen tiedekunta, Center for Life Course Health Research; Primary Health Care Center, Tornio, Finland; Karolinska Institutet, Division of Family Medicine and Primary Care Aro, Pertti; Arokero OY Walker, Marjorie; University of Newcastle, Professor of Anatomical Pathology Agreus, Lars; Karolinska Institutet, Division of Family Medicine and Primary Care Johansson, Sven-Erik; Karolinska Institutet, Division of Family Medicine and Primary Care Jones, Michael; Macquarie University, Psychology Talley, Nicholas; University of Newcastle, Faculty of Health and Medicine; University of Newcastle, Priority Research Center for Digestive Health and Neurogastroenterology; Australian GI Research Alliance
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Duodenal eosinophilia is associated with functional dyspepsia and new onset gastro-oesophageal reflux disease

Short title: Duodenal eosinophilia, functional dyspepsia and gastro-oesophageal reflux disease

Jukka Ronkainen[1,2,3], Pertti Aro[4], Marjorie Walker[5], Lars Agréus[3,5], Sven-Erik Johansson[3], Mike Jones[6] and Nicholas J Talley[,3,5].

[1]Center for Life Course Health Research, University of Oulu; [2]Primary Health Care Center, Tornio, Finland; [3]Division of Family Medicine and Primary Care, Karolinska Institutet, Stockholm, Sweden; [4]Arokero OY, Tornio, Finland; [5]Faculty of Health and Medicine, University of Newcastle, Priority Research Center for Digestive Health and Neurogastroenterology, Callaghan, NSW, and Australian GI Research Alliance (AGIRA), Australia; [6]Psychology Department, Macquarie University, NSW, Australia.

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Abbreviations: 95% CI – 95% Confidence Interval, EPS – Epigastric pain syndrome, FD - Functional dyspepsia, *H. pylori* - *Helicobacter pylori*, GORD – Gastro-oesophageal Reflux Disease, NSAID – non-steroidal anti-inflammatory drug, OR – odds ratio, PDS – Post prandial distress syndrome, PPI – Proton Pump Inhibitor

Keywords: Duodenal eosinophilia; Functional Dyspepsia; Gastro-oesophageal Reflux Disease.

Address for correspondence: Dr. Jukka Ronkainen, Ylipurrantie 17, SF-95410, Tornio Finland. Tel: +358 (0)40 5092259. E-Mail: jukka.ronkainen@fimnet.fi

Disclosures

The authors have nothing to disclose.

Writing Assistance

No writing assistance was provided.

Author Contributions

Jukka Ronkainen: Study design, performing the study, data collection and analysis, writing and revising article, guarantor of the article.

Pertti Aro: Study design, performing the study and collecting and analyzing the data, writing and revising the article.

Marjorie M Walker: Collecting and analyzing the data, revising the article.

Lars Agréus: Study design, supervising the study, writing and revising the article.

Sven-Erik Johansson: Analyzing the data, revising the article.

Mike Jones: Analyzing the data, revising the article.

Nicholas Talley: Original hypothesis, study design, supervising the study, writing and revising the article.

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Summary

Background: It is unexplained why functional dyspepsia and gastro-oesophageal reflux disease (GORD) overlap more than expected by chance. Postprandial distress syndrome has been linked to impaired gastric fundic accommodation which may induce increased transient lower oesophageal sphincter relaxations and consequent GORD. Duodenal eosinophilia has been linked to functional dyspepsia and postprandial distress syndrome.

Aims: We aimed to identify if there is an association between duodenal eosinophilia in functional dyspepsia and symptoms of GORD and whether postprandial distress syndrome or epigastric pain syndrome are associated with new onset GORD.

Methods: Participants (n=1000) were randomly selected from the national Swedish population register and surveyed by questionnaires and esophagogastroduodenoscopy in 1999-2001. All eligible subjects (n=887) were invited to a follow-up study in 2010 (response rate 79%). In a case-control study of 213 subjects (functional dyspepsia vs. healthy controls), histology from the duodenum was evaluated at baseline and the possible association of eosinophilia to new onset GORD symptoms was analysed.

Results: Functional dyspepsia (OR 7.6; 95% CI 2.93-19.4, P<0.001) and postprandial distress syndrome at baseline (OR 9.0, 95% CI 3.36-24.0, P<0.001) were associated with an increased risk of GORD at follow-up. Eosinophilia in the second part of duodenum only was independently associated with an increased risk of GORD among those with functional dyspepsia (OR 4.2; 95% CI 1.2-4.77, P=0.024) and postprandial distress syndrome at baseline (OR 6.0; 95% CI 1.50-23.6, P=0.011), respectively.

Conclusions: Duodenal eosinophilia is associated with up to six fold increased risk of GORD at 10-year follow-up in those with functional dyspepsia and postprandial distress syndrome at baseline. Duodenal eosinophilia may explain the link between GORD and functional dyspepsia, suggesting subsets of functional dyspepsia and GORD may be part of the same disease spectrum.

Introduction

Functional dyspepsia (FD) refers to unexplained pain or discomfort in the upper abdomen.[1] Gastro-oesophageal reflux disease (GORD) is perceived as a separate disease, defined as the reflux of gastric contents into the oesophagus causing troublesome symptoms, and may lead to complications including oesophagitis and Barrett's oesophagus.[2] Both FD and GORD are common in the general population and in those seeking health care around the world, each with a similar prevalence of around 20%.[3-5] Both conditions have a major negative impact on health related quality of life, and result in substantial costs for those affected and for society.[6-11] Although FD and GORD are considered separate and distinct disorders, it has been repeatedly observed that they overlap more than expected by chance (up to 60% of those affected) and this link remains largely unexplained.[12-14] One subtype of FD, postprandial distress syndrome (PDS) is associated with impaired gastric fundic accommodation; fundic disaccommodation may increase transient lower oesophageal sphincter relaxations (TLESRs) which may potentiate GORD.[15-17] The link between FD and GORD, however, is poorly understood and it is unknown whether PDS, in the absence of GORD at presentation, can predict future development of GORD.

Increased duodenal eosinophilia has been linked to FD worldwide.[18-22] It has been postulated that in those with a genetic predisposition to innate immune activation, an allergen such as wheat protein or infection may lead to antigen presentation, epithelial barrier disruption and an innate immune type 2 helper T-cell response, in which eosinophils in excess may degranulate to cause inflammation and neuronal sensitivity.[3,23-25] This may result in cytokine release with circulating homing T cells leading to altered gastroduodenal function, which may explain why symptoms are related to meals.[3,23,26] Similarly in GORD, a cytokine mediated pathogenesis has been suggested.[27,28]

The aim of this population based follow-up study was to identify prospectively if there is an association between duodenal eosinophilia and FD and its subtypes (postprandial distress syndrome and epigastric pain syndrome (EPS) as defined by the Rome III definition[1]) and new onset gastro-oesophageal reflux disease.

Methods

At baseline, the participants (n=3000) were randomly selected from the national Swedish population register and surveyed in 1998-2001 by a validated abdominal symptom questionnaire (ASQ).[29,30] Of these, 1000 individuals were randomly selected for an oesophagogastroduodenoscopy and a more comprehensive ASQ and medical history in 1999-2001 – the Kalixanda Study.[30,31] All eligible from the cohort with endoscopies performed at baseline (n=887) were invited for a 10-year follow-up study in 2010 with the comprehensive ASQ and medical history.[32] A case-control study on all available FD cases with histological evaluation of the duodenum at baseline (n=89) vs. healthy controls (n=124) was performed.

The Kalixanda study was approved by the Umeå University ethics committee (dnr 98-99, §156/98) and the follow-up study by the ethical approval committee of the Karolinska Institutet (2010/576-31/1) and conducted in accordance with the revised Declaration of Helsinki in 1998.

Endoscopy

The upper oesophagogastroduodenoscopies at baseline were undertaken by three experienced endoscopists, in the two clinics (Kalix and Haparanda), which gave sole medical coverage to the area. Internal validity was assessed by means of consensus sessions before the initiation of the basic study. A predefined endoscopy protocol was applied. Prior to endoscopy the endoscopists were blinded to the medical history and individual symptoms. Only topical anaesthesia was applied.

Oesophageal mucosal breaks (erosive oesophagitis) were graded according to the Los Angeles classification system.[33] The diagnosis of Barrett's oesophagus was based on the endoscopic finding of suspected columnar lined oesophagus in the distal oesophagus, confirmed histologically by the presence of specialised intestinal metaplasia in the oesophageal biopsy specimens. Peptic ulcer was

defined as a mucosal break at least 3 mm in diameter, with or without a necrotic base in the middle of the lesion, in either the stomach (gastric) or the duodenum (duodenal).[34,35]

Definitions of dyspepsia, gastro-oesophageal reflux, irritable bowel syndrome, anxiety and depression

Functional dyspepsia (FD) was defined based on the Rome III definition: weekly bothersome postprandial fullness or early satiation, or epigastric pain and/or epigastric burning with no findings of oesophagitis, peptic ulcer, celiac disease or cancer, and no evidence of other structural disease at endoscopy that was likely to explain the symptoms. FD, according to the Rome III definition,[1] was divided into:

1. Postprandial Distress Syndrome (PDS) consisting of bothersome postprandial fullness and/or early satiation.
2. Epigastric Pain Syndrome (EPS) consisting of pain or burning localized to the epigastric area and not generalized or localized to other abdominal or chest regions, and not relieved by defecation. Overlap between PDS and EPS was allowed in line with the Rome III definition. The presence of heartburn/gastro-oesophageal reflux or irritable bowel syndrome (IBS) did not exclude the diagnosis of FD.[1]

The ASQ was designed prior to the Rome era but the questionnaire has been updated to meet both the Rome II and III criteria and it measures all of the Rome III criteria aside from the symptom onset criterion (3 vs. 6 months).[29,36]

Gastro-oesophageal reflux disease symptoms were defined as troublesome heartburn and/or acid regurgitation over the past three months, which is in line with the Montreal definition.[2]

Irritable bowel syndrome IBS was defined as any of troublesome abdominal pain located at any site plus concomitant bowel habit disturbances (constipation, diarrhea, or alternating constipation and diarrhea). [29,36]

Anxiety and depression were defined by a score of 11 or higher in the hospital anxiety and depression scale.[37]

Biopsies

Oesophageal and gastric biopsies were taken as previously described. [38] Biopsies were also taken from the duodenal bulb and from the second (descending) part of the duodenum. Biopsies were stained with haematoxylin and eosin, and gastric biopsies additionally stained with Warthin-Starry for detection of *H. pylori*. Current *H. pylori* infection was identified if histology and/or culture detected the bacteria.[39] Eosinophils were quantified by counting the number per high-power field (magnification x40); 5 high-power fields were selected randomly in each section. The sum, mean, and median over the 5-field counts then were calculated in every subject, the pre-specified cut off being the mean, 23 eosinophils in the bulb and 24 eosinophils in the second part of duodenum.[18] The cut off for gastric eosinophilia was also the mean, 11 eosinophils in all three locations: cardia, corpus and antrum. Eosinophilic oesophagitis was defined by 15 or more eosinophils/high-power field in histology in oesophageal biopsies from the Z-line and/or 2 cm above the Z-line.

The study was performed in 213 subjects based on all available FD cases with histological evaluations of the duodenum at baseline vs. healthy controls, comprising 89 cases with FD and 124 controls without FD, irritable bowel syndrome, GORD or any gastrointestinal disease found in endoscopy or medical records, mean age 62.3 years, 66% female (Figure 1). The subjects included

in this study were similar to the remaining subjects in the population-based follow-up cohort who completed the postal questionnaire (Table 1).

Statistics

Data were analysed by chi square test and Fishers exact test (when expected frequency was < 5 in a cell) for testing significance in univariate analyses and in continuous variables by testing equal variance and by the t-test for analysing significances in means. Predictors for change in presentation of FD, PDS, and EPS to new onset GERD were analysed in separate logistic regression analyses adjusting for age, gender and anxiety and including duodenal eosinophilia in the final model. Categorized BMI[40], use of proton pump inhibitors (PPIs), use of H_2-receptor antagonists (H2RAs), use of anti-allergy medication, use of non-steroidal anti-inflammatory drugs (NSAIDs) and/or aspirin, smoking (yes/no), *H. pylori* infection, anxiety, depression and use of alcohol (>100g/week) were tested in univariate analysis and only statistically significant variables were introduced into the model. Due to non-linearity, age was dichotomized with the cut off at 60 years of age. Variables above were chosen according to our *a priori* hypothesis and their clinical significance. The goodness-of-fit was tested by Pearson χ^2 test and if the P-value was greater than 0.05 the model was judged to fit the data. P-values were two tailed and the alpha level of significance was set at 0.05. The prevalence is shown as a percentage with a 95% confidence interval (CI). The Intercooled STATA 11 program was used for analyses.[41]

Results

Of the baseline study population (n=3,000), 2,860 were eligible for inclusion. The overall response rate to the postal ASQ was 74.2 % (n=2,122). According to protocol, 1,364 responders to the ASQ eligible for upper endoscopy were invited in random order for an EGD with a response rate of 73.3% (n=1,000). Ten years after the baseline endoscopy, 887 subjects were eligible for the follow-up study and 703 out of those answered the postal questionnaires (response rate 79.3%), mean age 63.2 years, 52% female (Figure 1).

Although the majority of FD cases in the 10-year follow-up were female (74/365 vs. 36/338, P=0.003), the smoking habits, use of alcohol, use of aspirin and/or NSAIDs, use of PPIs and prevalence of *H. pylori* infection did not differ between the study group (n=213) and the 703 subjects at 10-year follow-up (Table 1).

In the FD case-control study of 213 subjects, the mean age did not differ between FD cases and controls at baseline (51.8 vs 52.3 years, P=0.76) or at follow up (60.8 vs 62.7 years, P=0.36).Of the 213 subjects, FD was reported by 89 subjects at baseline (71 PDS and 27 EPS, overlap in 9) and by 41 at follow-up (39 PDS and 6 EPS, overlap in 4 (Figure 2).

Concomitant GORD symptoms (with no oesophagitis or other organic disease at baseline) were reported by 54 FD subjects (25.4%, 95% CI 19.5-31.2) at baseline and 68 subjects (31.9%, 95% CI 25.6-38.2) reported any GORD symptoms at follow-up (overlap in 44), of whom 24 had developed new onset GORD at follow-up (Figure 2).

Duodenal eosinophil counts were increased in subjects with FD without concomitant IBS or GORD at baseline. The mean eosinophil counts in the bulb were 32.2 (SD, 23.4) compared with 22.6 (SD, 23.3) in controls (P=0.08), and in the second part of duodenum 32.0 (SD, 17.0) compared with 22.4 (SD, 13.6) in controls (P=0.004), respectively. Any duodenal eosinophilia (Figure 3) was found in 78 subjects in the bulb and in 84 subjects in the second part of duodenum (in 46 subjects, both in the bulb and in the second part of duodenum, P<0.001) at baseline.

Anxiety at baseline was associated with duodenal eosinophilia in the second part of duodenum (8/78 vs. 1/124, P=0.002). There were only two subjects with depression at baseline. *H. pylori* infection (34/116 vs.37/97, P=0.173, use of PPIs (7/116 vs. 6/97, P>0.9), H2RAs (4/116 vs. 1/97, P=0.379) or anti-allergy medication (6/116 vs. 7/97, P=0.576) were not associated with duodenal eosinophilia. In addition, categorized BMI, use of NSAIDs and/or aspirin, smoking, and use of alcohol were not associated with duodenal eosinophilia (data not shown).

None of the study subjects had eosinophilic oesophagitis and only one had any oesophageal eosinophilia. Duodenal eosinophilia was not associated with gastric eosinophilia (OR=0.18, 95 CI 0.02-1.70, P=0.135).

Duodenal eosinophilia was associated with FD without concomitant IBS or GORD (n=22) (bulb: 15/56 vs. 7/90, P=0.002, the second part of duodenum: 16/60 vs. 6/86, P=0.001).
Eosinophilia in the second part of duodenum was associated with PDS without concomitant IBS or GORD (bulb: 10/76 vs. 7/90, P=0.065; the second part of duodenum: 12/60 vs.5/86, P=0.009).
Duodenal eosinophilia was not associated with EPS without concomitant IBS or GORD (bulb: 6/56 vs. 3/90, P=0.086, the second part of duodenum: 6/60 vs. 3/86, P=0.161). Neither FD with

concomitant GORD nor FD with concomitant IBS was associated with duodenal eosinophilia at baseline (data not shown).

Duodenal eosinophilia independent of FD status was not associated with GORD at baseline (bulb: 19/54 vs. 59/159, P=0.800, the second part of duodenum: 20/54 vs.64/159, P=0.676) or with GORD at follow-up (bulb: 27/68 vs.51/145, P=0.522, the second part of duodenum: 23/68 vs. 61/145, P=0.251) or new onset GORD (bulb: 12/24 vs. 66/189, P=0.149, the second part of duodenum: 10/24 vs. 74/189, P=0.812).

Predictors of GORD

Duodenal eosinophilia in the second part of duodenum (bulb: OR 2.1, 95% CI 0.66-6.56, P=0.213, the second part of duodenum: OR 4.2, 95% CI 1.21-14.8 P=0.024) was associated with a change from FD with no concomitant GORD (n=35) at baseline to new onset GORD at follow-up (14/24 vs. 21/135, OR 7.6; 95% CI 2.93-19.4, P<0.001) (Table 2).

Eosinophilia in the second part of duodenum (bulb: OR 1.6; 95% CI 0.50-4.86, P=0.443, the second part of duodenum: OR 6.0; 95% CI 1.50-23.6, P=0.011) was associated with a change from PDS with no concomitant GORD (n=29) at baseline to new onset GORD at follow-up (13/24 vs. 16/135, OR 9.0, 95% CI 3.36-24.04, P<0.001) (Table 2), (Figure 4).

There were only 3 subjects with change from EPS with no concomitant GORD at baseline to new onset GORD (3/24 vs. 8/135, OR 2.5, 95% CI 0.60-10.5, P=0.211) and there was no association with duodenal eosinophilia in these subjects (P>0.9).

Discussion

This is a unique 10-year prospective follow-up study on a random sample of the general population with endoscopy, abdominal symptom questionnaires and medical record review performed at baseline, and validated postal questionnaires and medical record review performed at follow-up. We found a significant association of duodenal eosinophilia in the second part of duodenum with new onset symptomatic GORD at 10 years in those with functional dyspepsia and especially PDS subtype at baseline. There were only 3 subjects with change from EPS with no concomitant GORD at baseline to new onset GORD, an inadequate number to draw conclusions.

New onset GORD symptoms in patients with FD originally free of reflux symptoms may be due to gastro-oesophageal reflux disease from an increased acid load bathing the lower oesophagus, or alternatively, may be a manifestation of functional heartburn secondary to the development of new onset oesophageal hypersensitivity. We suggest that all of these mechanisms are feasible.[42]
The pathogenesis of FD is considered multifactorial including associations with psychological distress, post-infectious gastroenteritis, and in a minority with *H. pylori* infection as documented by symptom resolution 6 to 12 months after eradication therapy, although dyspeptic symptoms associated with *H. pylori* may also be considered as a separate entity. [3,43-45] However, at least for a major subset with postprandial distress syndrome, a new pathophysiological hypothesis has emerged namely low grade duodenal inflammation usually characterized by an innate immune response with increased eosinophils and in some cases excess mast cells.[23,46,47]

Several studies around the world have confirmed an association of duodenal eosinophilia with FD not linked to *H. pylori* or oesophageal eosinophilia.[18-22,48] Furthermore, duodenal permeability is increased in FD as are submucosal neuronal structural and functional changes that correlate with

inflammation.[49] FD is also associated with circulating homing T-cells, a marker of small intestinal inflammation, and increased cytokines.[26]

Similarly in GORD, a cytokine mediated pathogenesis has been suggested based on the finding of increased intraepithelial T cells in the oesophagus after stopping PPI in patients with established reflux oesophagitis.[27] The duodenum is the gateway to the small intestine and a master controller of gastroduodenal function regulating gastric emptying and gastric accommodation via neural and endocrine pathways which ensure chyme reaches the small intestine in a controlled fashion to promote absorption.[23] Therefore, duodenal hypersensitivity secondary to inflammation, which occurs in about 40% of those with FD may alter fundic relaxation.[23] Further, impaired fundic relaxation and/or delayed gastric emptying potentially can lead to increased transient lower oesophageal sphincter relaxations (TLESRs) inducing true pathological gastro-oesophageal reflux.[15] This interpretation is further supported by the observation of increased pathological acid reflux by oesophageal pH testing in 20% to 30% among otherwise "gold standard" FD cases.[50,51]

We have earlier shown that GORD symptoms persisted in approximately 81% in the short term (range 1 to 6 months) and were more stable also in the long term than FD or IBS symptoms.[12,32] We also showed that anxiety at baseline was associated with an 8-fold risk for FD in the next 10 years. An association between anxiety and duodenal eosinophilia has been described in children[52] and in this study anxiety was associated with duodenal eosinophilia in the second part of duodenum in adults. Overall our results support the concept that the mucosal immune system can regulate gut-brain communication perhaps inducing PDS although the exact pathways including the role of the duodenal microbiome remain to be elucidated.[24,53]

In our study there was good concordance for duodenal eosinophilia in the bulb and in the second part of duodenum (P<0.001). Notably, PDS without concomitant GORD was associated only with eosinophilia in the second part of duodenum, while EPS without concomitant GORD was not associated with duodenal eosinophilia at baseline, suggesting that these subgroups of FD differ in pathogenesis.[14] For example, we postulate that acid may play a role in inducing duodenal eosinophilia in the bulb because of its closer proximity to high loads of gastric acid bathing the bulb. The fact that eosinophilia in the second part of duodenum only was associated with the transformation of FD and especially PDS to new onset GORD further supports the concept that the duodenum is a primary driver of the pathogenesis. Another report from Australia also found that early satiety was associated with eosinophilia in the second part of duodenum but they found lower eosinophil counts in the bulb compared to the second part of duodenum.[19] Our finding that there may be a role for duodenal eosinophilia as the link between FD and GORD, possibly via a cytokine mediated pathogenesis, may have potential therapeutic implications in the near future.[3,27] Damping down duodenal inflammation with targeted anti-inflammatory therapy for example may improve impaired fundic accommodation and pathological acid reflux as well as gastro-oesophageal reflux and dyspepsia symptoms in some cases, a novel hypothesis that now needs to be investigated.

In an independent 10-year follow-up study, FD symptoms were more common in females and younger subjects.[54] At 10 years those with FD were younger than controls in the present study but in the case-control study this was significant only in males (data not shown). This difference may be due to gender specific biological differences in gastrointestinal function but whether sex-hormone actions on intestinal function, or whether gender specific processing of pain in the central nervous system or health behaviour issues are of importance is unclear.[55,56] As some gastrointestinal functional disorders may decline with advancing age, we speculate this phenomenon may occur

secondary to intestinal immune activation declining with age, a hypothesis warranting further studies.

The strengths of this study are that a random sample of a true general population was prospectively studied. The response rate was high at all time points. Both endoscopists and pathologists were blinded to symptoms and medical history before the examinations, and validated questionnaires were used.

A limitation of the study was the fact that we only had histology of the duodenum available at baseline, not at follow-up. Another potential weakness is that a pH impedance study was not performed to confirm acid or non-acid reflux and differentiate visceral hypersensitivity and functional heartburn due to the fact that participants came from the population and were not patients. However, GORD diagnosis was based on the widely accepted Montreal definition. In addition, use of PPIs, H_2RAs and/or anti-allergy medication may be confounders but their use was uncommon based on the medical records, and medication use was controlled for in the statistical analyses. Duodenal eosinophilia in IBS was not demonstrated in this population[46] and in this cohort there was no increased duodenal eosinophilia in those with concomitant FD and IBS. Although the original random population sample was large, the CIs in subgroups of FD, especially EPS were relatively wide and no conclusion about the relationship of EPS and GORD can be drawn. Notably ORs were high and statistically significant and were in the anticipated direction based on our *a priori* hypothesis. While we modified the Rome III definition slightly in terms of the symptom history only (3 months rather than 6 months, Rome III FD definitions included in the ASQ), we do not believe this would have had any major impact.

In conclusion, duodenal eosinophilia is associated with up to six fold increased risk of new onset GORD at 10-year follow-up in those with FD and PDS at baseline, but not with EPS. PDS but not

EPS has been linked to impaired gastric accommodation. Although causality cannot be confirmed in a population-based cohort study, we hypothesize duodenal eosinophilia may thus explain the link between GORD and FD via impaired gastric accommodation and increased transient lower oesophageal sphincter relaxations.[15,57] Our novel data support the concept FD and GORD are part of the same disease spectrum.

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Table 1. Baseline characteristics and prevalence of possible confounders in all 703 subjects at 10-year follow-up and in the functional dyspepsia case-control study of 213 subjects with duodenal eosinophil counts at baseline.

	All 703 at follow-up n, %	Case-control study n=213 n, %	P-value
Mean age (years)	63.2	62.1	0.36
Female	365, 51.9%	141, 66.2%	**0.001**
Body mass index, mean†	26.3	26.1	0.52
Use of NSAIDs or aspirin	122, 17.4%	37, 17.40%	>0.90
Proton pump inhibitor	36, 5.1%	13, 6.1%	0.56
H_2-receptor antagonists	21, 3.0%	5, 2.4%	0.81
Anti-allergy medication	43, 6.1%	13, 6.1%	>0.90
Smoking	117, 16.6%	40, 18.8%	0.52
Alcohol	86, 12.2%	23, 10.8%	0.57
H. pylori	214, 30.4%	71, 33.3%	0.42
Anxiety‡	21, 3.1%	9, 4.5%	0.40
Depression§	8, 1.2%	2, 1.0%	>0.90

NSAIDs = Non-Steroidal Anti-inflammatory Drugs

H_2-receptor antagonists = Histamine H2 receptor antagonists

H. pylori = *Helicobacter pylori,* positive by histology or culture.

†Data available in 697/703 and 211/213.

‡Data available in 671/703 and 202/213.

§Data available in 673/703 and 203/213.

Alcohol>100g/week

Table 2. Association of duodenal eosinophilia in the second portion of the duodenum (D2) with change of functional dyspepsia (FD), postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) at baseline to new onset symptomatic GORD at 10-year follow-up

Predictor	**Univariate analysis[1]**	**Adjusted for age and gender[2]**	**Final model[3]**
FD to GORD	**P=0.049**	**3.13 (1.00-9.80), P=0.050**	**4.22 (1.21-4.77), P=0.024**
PDS to GORD	**P=0.023**	**4.06 (1.19-13.3), P=0.025**	**5.95 (1.50-23.63), P=0.011**
EPS to GORD	P>0.9	0.61 (0.05-6.94), P=0.691	1.35 (0.08-22.19), P=0.835
Age	P=0.274	1.33 (0.73-2.41), P=0.346	1.66 (0.87-3.16), P=0.124
Gender	P=0.193	0.66 (0.36-1.22), P=0.182	0.67 (0.35-1.29), P=0.236
Anxiety	**P=0.002**	n/a	**16.4 (1.92-139.05), P=0.010**

[1]Univariate analysis by chi square test or Fishers exact test (when expected frequency was < 5 in a cell)

[2]Adjusted for age and gender, age dichotomized at 60 years of age

[3]Selected based on variables tested significant in univariate analysis and by testing the goodness-of-fit by Pearson chi[2] test: P=0.794

FD = functional dyspepsia, PDS = postprandial distress syndrome, EPS = epigastric pain syndrome, GORD = gastro-oesophageal reflux disease

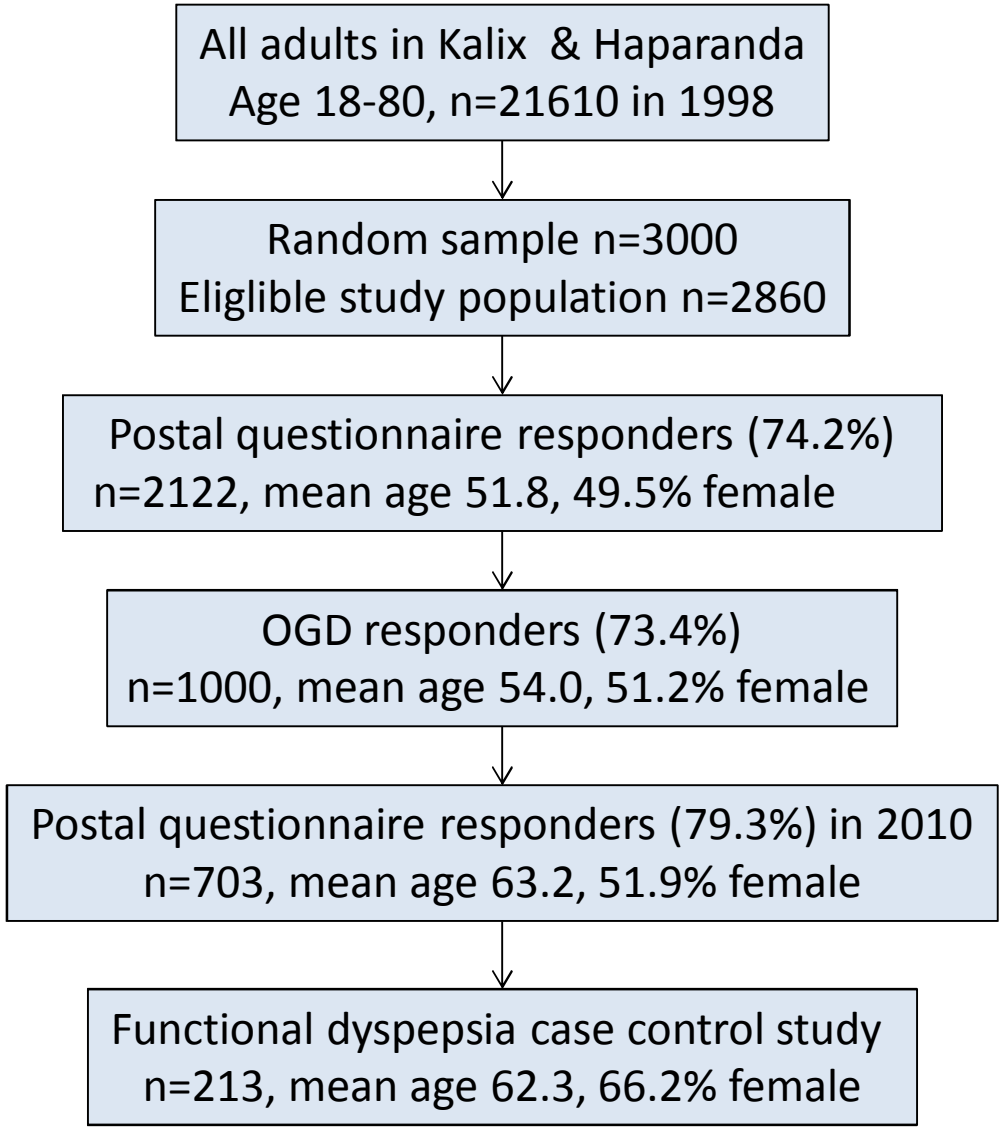
Figure 1. Flow chart of the population-based functional dyspepsia 10-year follow-up study.

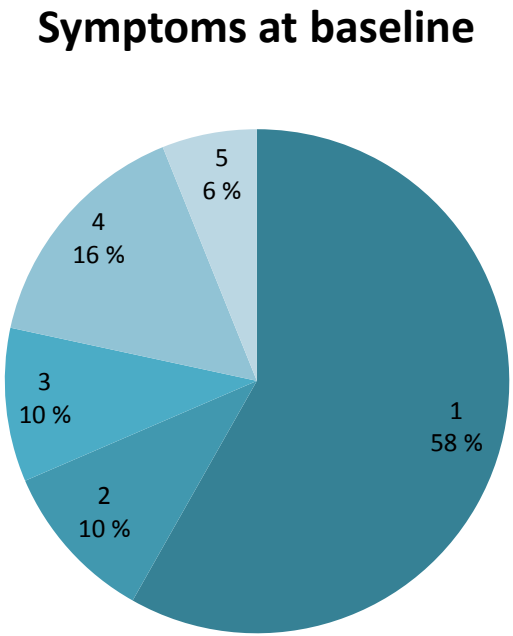
Figure 2. Symptoms at baseline and at 10-year follow-up.

Figure 3. Normal duodenal villous architecture (A), and eosinophil clusters in lamina propria >24/5 HPF (B)

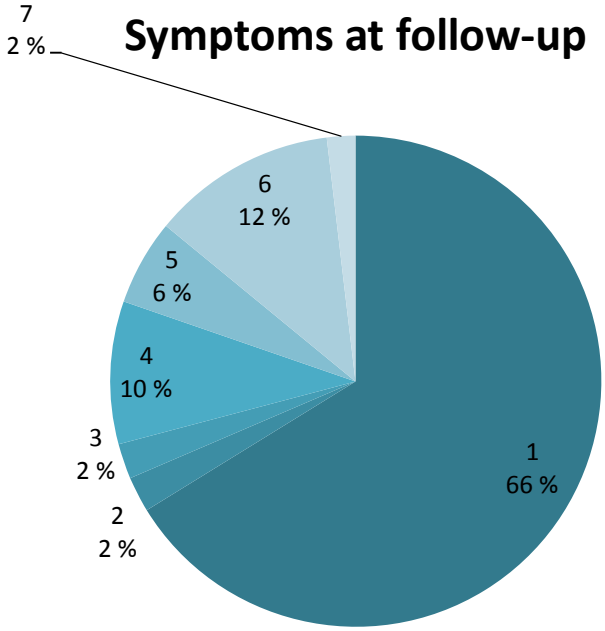
Figure 4. Functional dyspepsia at baseline and gastro-oesophageal reflux disease at 10-year follow-up.

The Kalixanda 10-year functional dyspepsia follow-up study





1. Healthy controls, n=124
2. FD, n=22
3. FD+GORD, n=21
4. FD+GORD+IBS, n=33
5. FD+IBS, n=13



1. No symptoms, n=141
2. IBS, n=5
3. IBS+FD, n=5
4. IBS+FD+GORD, n=20
5. FD+GORD, n=12
6. GORD, n=26
7. FD, n=4

(A) Normal villous architecture, no increased intraepithelial lymphocytes,
(B) Eosinophil clusters in lamina propria >24/5 HPF, circled.
Original magnification x40

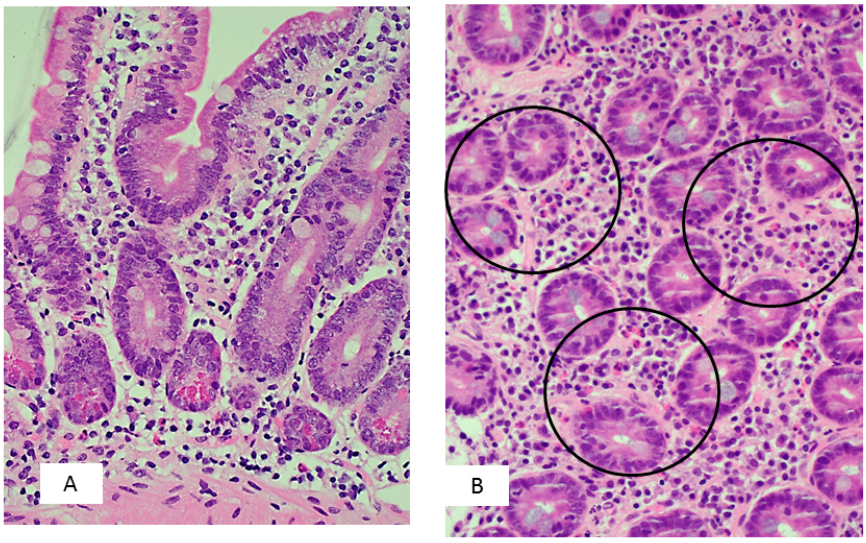
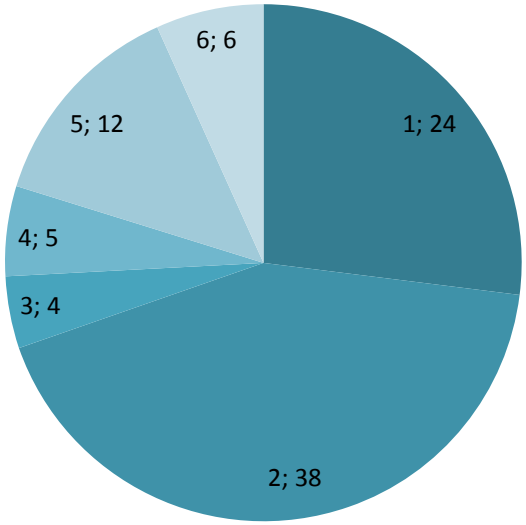


Figure 3. Normal duodenal villous architecture (A), and eosinophil clusters in lamina propria >24/5 HPF (B)

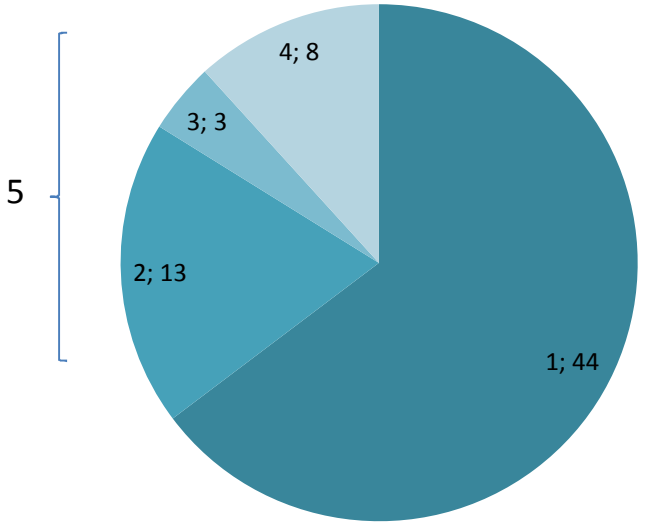
254x190mm (96 x 96 DPI)

FD at baseline
n=89



1. PDS, n=24
2. PDS, GORD, n=38
3. PDS, EPS, GORD, n=4
4. PDS, EPS, n=5
5. EPS, GORD, n=12
6. EPS, n=6

GORD at follow-up
n=68



1. GORD at baseline and at follow-up, n=44
2. PDS to incident GORD, n=13
3. EPS to incident GORD, n=3
4. Incident GORD, n=8
5. 2+3+4, n=24, New onset GORD

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract OK	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction OK		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods OK		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(*b*) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how matching of cases and controls was addressed
		(*e*) Describe any sensitivity analyses
Results OK		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion OK		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information OK		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.