Cohort Profile: The Christchurch IBS cOhort to investigate Mechanisms FOr gut Relief and improved Transit (COMFORT)

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Keywords

Irritable bowel syndrome · Functional gastrointestinal disorders · Cohort profile · Observational study · Case control study

Abstract

Background and aims: This cross-sectional observational case-control study was initiated in July 2016 with the aim of increasing an understanding of the underlying disease mechanisms in functional gastrointestinal disorders (FGIDs) including irritable bowel syndrome (IBS), functional diarrhoea (FD), and functional constipation (FC). Specific areas of interest include the effect of food, microbiome, host and microbial genetics, metabolome, and psychological variables on unexplained chronic gastrointestinal (GI) symptoms. **Methods:** This study recruited consecutive patients

who were attending one of two endoscopy centres in Christchurch, New Zealand, for colonoscopy and a subgroup of participants from the general public who did not undergo colonoscopy. Participants with known GI disease other than an FGID were excluded. Those with symptoms were recruited as cases, whilst those without symptoms were recruited as controls. In the days prior to preparation for colonoscopy, or an agreeable time for those not undergoing colonoscopy, demographic, symptom, psychological, dietary, and health data were collected in addition to biological samples

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(breath, faeces, blood, and urine). Colonic biopsies were taken at the time of colonoscopy from participants in the colonoscopy subgroup. **Results:** Between July 2016 and December 2018, 349 participants were recruited, 315 of whom completed the study, 220 participants were from the colonoscopy subgroup, and 95 from the non-colonoscopy subgroup. This included 129 controls and 186 cases (57 IBS-diarrhoea predominant, 30 IBS-constipation predominant, 41 IBS-mixed, 42 FC, and 16 FD). The mean age of FGID cases was 53.4 years and controls 54.4 years. Cases (149/186, 80.1%) and controls (57/72, 55.8%) were predominantly female. Education levels were similar across the cohort. Smoking and alcohol rates were also similar. Biological samples were collected as planned from participants. **Conclusions:** The COMFORT cohort is a unique clinical cohort of FGID cases and controls with a wide range of demographic, dietary, clinical, psychological, and health data in addition to biological samples. Future research will aim to use a systems biology approach to establish the potential role of diet, host-microbiome interactions, and other factors in the pathogenesis of FGIDs.

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Introduction

In clinical practice, patients commonly consult with unexplained chronic gastrointestinal (GI) symptoms referred to as functional gastrointestinal disorders (FGIDs); in these conditions, no obvious physical cause is identified on routine testing [1]. One of the most common FGIDs is the irritable bowel syndrome (IBS), which is associated with significant health care costs and decreased quality of life [2] and is also one of the most common causes of visits to primary care physicians and gastroenterologists [2–4]. IBS predominantly affects women and is often diagnosed in those aged 35 years or younger [5, 6]. While prevalence differs between countries, IBS global prevalence is estimated to be 11% [7].

IBS is characterised by recurrent abdominal pain associated with a change in the frequency and/or form of bowel motions in the absence of a known organic cause [1]. These symptoms exist on a spectrum, with IBS being subtyped into constipation-, diarrhoea-, or mixed phenotype-predominant (IBS-C, IBS-D, and IBS-M, respectively), depending on the frequency and form of individual patients' bowel motions [1, 8]. Currently, there are no reliable biomarkers for the diagnosis of IBS [9]; the current “gold standard” diagnostic framework, the Rome IV criteria, uses the presence of GI symptoms typical of

IBS in the absence of organic disease as a basis for diagnosis [1].

Current scientific consensus has led to the definition of IBS (and other FGIDs) as a dysregulation of the brain-gut axis (BGA) [10]. However, the aetiology of IBS is complex and ultimately unknown. Multiple biological systems have been implicated in IBS pathophysiology [11]: differences have been discovered between IBS patients and healthy controls in inflammation pathways, the immune system, the GI epithelial barrier, the GI microbiota, the hypothalamic-pituitary-adrenal axis, cognitive areas of the central nervous system, and the enteric nervous system [12]. These differences have been presumed to lead to the manifestation of classical IBS symptoms such as visceral hypersensitivity (linked to recurrent abdominal pain) and altered GI motility [13–19].

Rationale for the Study

Due to the heterogeneous presentation of IBS symptoms between patients and sometimes within the same patient over time, treatments for IBS have historically been less than satisfactory. These have included lifestyle changes, fibre supplementation, behavioural therapy, antidepressants, and antispasmodics [20–23]. While these treatments may reduce IBS symptoms in some patients, at present there is no known cure for IBS, frustrating clinicians and patients alike.

Research into the aetiology of IBS has traditionally focused on only one or two potential aspects of pathogenesis; however, due to recent technological advances, researchers are increasingly moving towards a systems biology approach to addressing the potential multifactorial aetiology of this disorder [24]. As such, the Christchurch IBS cOhort to investigate Mechanisms FOr gut Relief and improved Transit (COMFORT) study was designed to capture and integrate a wide range of biological, clinical, demographic, psychological, and dietary data in order to study the pathophysiology of IBS. As a prospective observational case control study, the COMFORT study aims to (1) develop a novel cohort specifically designed to investigate a wide range of variables and their effect on IBS pathogenesis, activity, and prognosis, (2) develop dietary profiles for those with correlations to IBS symptomology in those with and without IBS, (3) correlate and integrate these dietary data with biological, clinical, and psychological variables, and (4) develop a data repository of biological specimens that will allow for prospective in vitro and ex vivo experiments for novel food-based interventions in IBS.

IBS				Other FGID	
1. Abdominal pain a) More than once a week b) Associated with change in frequency and/or form of stools				1. Symptomatic 2. Do not meet the criteria for IBS	
IBS-D 1. >25% stools Bristol stool chart 6–7 2. <25% stools Bristol stool chart 1–2	IBS-C 1. >25% stools Bristol stool chart 1–2 2. <25% stools Bristol stool chart 6–7	IBS-M 1. >25% stools Bristol stool chart 1–2 2. >25% stools Bristol stool chart 6–7	IBS-U 1. Meet diagnostic criteria for IBS 2. Stool cannot be classified into IBS-D, IBS-C, or IBS-M	FC 1. Must include 2 of the following: a) >25% straining during defecations b) >25% stools Bristol stool chart 1–2 c) >25% sensation of incomplete evacuation d) >25% sensation of anorectal blockage e) >25% manual manoeuvres to facilitate defecations f) <3 complete spontaneous bowel motions per week 2. Loose stools rare without laxative use	FD 1. >25% stools Bristol stool chart 6–7 2. Stool without predominant abdominal pain or bloating
1. Criteria fulfilled for the last 3 months 2. Symptoms onset more than 6 months prior					

Fig. 1. Summary of the Rome IV diagnostic criteria.

Methods

Setting, Location, and Relevant Data

Participants who were undergoing colonoscopy at one of two endoscopy units in Christchurch, New Zealand, were referred by their treating physician prior to their procedure (colonoscopy subgroup). Other potential participants who responded to advertisements and were not undergoing colonoscopy were also recruited (non-colonoscopy subgroup). Potential participants were contacted by a member of the research team with further information about participation in the study. The eligibility of participants (18–70 years inclusive) was assessed by the research team, using the Rome IV criteria (summarised in Fig. 1) [1], which was used to diagnose bowel disorder (IBS, functional diarrhoea [FD], or functional constipation [FC]) cases. The presence of other FGIDs was not assessed. Prospective participants with “red flag” symptoms indicative of organic disease including blood in the stool, nocturnal symptoms, unexplained weight loss, and anaemia were excluded from the study, as were participants who had a history of GI diseases or abdominal surgery, pregnancy, or were unable to give informed consent. Participants undergoing colonoscopy were also excluded if significant organic bowel disease was discovered. Participants who did not meet exclusion criteria and who had no significant GI symptoms (functional or otherwise) were enrolled in the study as controls. Controls from the colonoscopy subgroup were participants who were undergoing colonoscopy but did not have GI symptoms or a history of an underlying significant health problem including GI disease. Many of these controls may have had a past history or family history of sporadic polyps or colorectal cancer, respectively, and were undergoing surveillance, but patients with familial polyposis syndromes were excluded. Eligible patients were enrolled in the study, and written informed consent was obtained from each participant to participate in the study prior to any study procedures being undertaken.

Collection and Processing of Samples and Data

Once written informed consent was obtained, an electronic case report form (eCRF) was generated for each participant, who was de-identified and given a unique 5 digit ID number. These, and all other information pertaining to participants, were stored physically or electronically according to ethical requirements. The research team organized the start date of the study individually with each participant to optimize the completion of questionnaires, the efficient return of biological samples, and the close timing of data and sample collection in those who underwent colonoscopy. Questionnaires and biological sample collection equipment were labelled with the participants’ 5-digit ID number and posted to the participant. The dietary data collection and questionnaires were completed over 3 days (Fig. 2). For those participants who were undergoing colonoscopy, all data and biological samples except colonic mucosal biopsies were collected before the commencement of bowel preparation (no later than 48 h prior to colonoscopy).

A wide range of variables were collected as part of the COMFORT cohort study procedures (Fig. 3). Biological samples collected included breath, blood, faeces, urine, and colonic biopsy tissue. A range of questionnaires were used to collect many clinical, demographic, and psychological variables. The Modified Hunter New England Survey [25] included Rome IV questionnaires [26] to classify participants as controls, cases, and sub-phenotypes, Short Form-12 for quality of life [27], medical history questions, and demographics, and the Hospital Anxiety and Depression Scale (HADS) [28]. The validated Structured Assessment of Gastrointestinal Symptoms (SAGIS) [29] evaluated a wide range of GI symptoms. The Economic Standard of Living Index-short form (ELSI-sf) [30] is a validated comprehensive measure of living standard in New Zealand. The Patient-Reported Outcomes Measurement Information System (PROMIS®) [31–33] tools for GI symptoms and emotional distress are validated tools for measuring

		Pre-enrolment ¹	Day 1 ²	Day 2 ²	Day 3 ²	Day 4 ³	Colonoscopy ⁴
Recruitment	Advertisements	×					
	Referral from Gastroenterologist	×					
Written informed consent from willing participants		×					
Modified hunter New England survey (ModHNES)	Rome IV		×				
	SF-12						
	Medical history						
	Hospital anxiety and depression scale (HADS)						
	Demographics						
Structured assessment of gastrointestinal symptoms (SAGIS)			×				
Economic living standards index – short form (ELSI-sf)			×				
Patient reported outcomes measurement information system – gastrointestinal (PROMIS-GI)					×		
PROMIS-emotional distress					×		
Food and symptoms times (FAST) diary			×	×	×		
Stool sample collection						×	
Urine sample collection						×	
Blood sample collection						×	
Breath sample collection						×	
Colonic mucosa sample collection							×

Fig. 2. Schedule of COMFORT cohort variables and visits. ¹Recruitment and informed consent was obtained from participants prior to their enrolment in the COMFORT study; subsequently, all paperwork and sample collection packs were delivered to the participant for completion at home. ²Day 1 of the study was the day participants commenced the FAST diary and completed the MODHNES, SAGIS, and ELSI-sf; the FAST diary was also filled

out on day 2 and day 3. The PROMIS-GI and PROMIS-emotional distress were also completed on day 3. ³On day 4 of the study, the participants collected their stool and urine sample at home and then met with the study team in order to collect blood and breath samples. Days 1–4 were consecutive days. ⁴Colonic mucosa biopsies were collected during colonoscopy (in the colonoscopy subgroup only).

these outcomes and can be compared to population norms. The Food and Symptom Times (FAST [34]) diary is a validated 3-day weighted food diary, which records all aspects of participants' diets and GI symptoms contemporaneously. This allows for temporal relationships between food and symptoms to be explored.

Stool and urine samples were collected on the last day of the FAST diary at participants' homes into 60 mL specimen containers provided to participants (Fig. 2). Samples were kept at 4°C and delivered to the research team within 24 h of collection at which time 58 mL of blood and 3 L of breath were obtained from the participant. Samples were initially processed at the University of Otago, Christchurch; volatile metabolites were extracted from the breath

samples onto a carbon trap within an hour of collection and stored sealed at 4°C. Stool and urine samples were kept at 4°C until they were sorted into 1 g aliquots and then snap frozen with liquid nitrogen within 24 h of collection. Eighteen millilitres of lithium heparin (LiH)-treated blood, 12 mL of ethylenediaminetetraacetic acid-treated blood, and 10 mL of untreated blood were centrifuged at 2,000 g, and plasma was distributed into 0.5 mL aliquots and stored at –80°C. Peripheral blood mononuclear cells (PBMCs) were extracted from the remaining 18 mL of LiH-treated blood, two-thirds of which were stored in RNA cell protect at –80°C and the last third in a solution of 90% foetal bovine serum in the gaseous phase of liquid nitrogen [35, 36]. In those participants who

Biological samples		Questionnaires	Fast diary
Blood	Metabolomics Neuromarkers PBMCs Immune markers DNA Biobank	Rome IV diagnostic criteria Short form-12 (SF-12) • Measurement of mental and physical quality of life Medical history and demographics Hospital anxiety and depression scale (HADS) • Validated measure of anxiety and depression symptoms Patient reported outcomes measures information system (PROMIS®) Gastrointestinal and emotional distress • Patient reported outcome for measurement of subjective symptoms Structured assessment of gastrointestinal symptoms scale (SAGIS) • Symptoms severity scale Economic living standard index – short form (ELSI-sf) • New Zealand specific measure of deprivation level	Food and symptoms times (FAST) diary • 3 day weighted diet diary with concomitant gastrointestinal symptoms scores • Records presence, severity, and duration of gastrointestinal symptoms
Breath	Volatile metabolites		
Faeces	Untargeted metabolites Targeted metabolites 16S DNA Metagenome DNA Biobank metagenome RNA Biobank		
Urine	Metabolomics Biobank		
Tissue	Histopathology Immunohistochemistry RNA expression Biobank		

Fig. 3. Biological samples and intended experiments, questionnaires and diet diary completed by each participant enrolled in the COMFORT study.

underwent colonoscopy and in those who consented for biopsies to be taken for research purposes, 8 colonic mucosa samples were collected from participants undergoing colonoscopy, four from the right colon and four from the left colon. Two biopsies from each side of the colon were stored in RNeasy® at -80°C , and the rest of the biopsies were fixed in formalin and then embedded in paraffin blocks.

Results

Recruitment began in July 2016 at the Christchurch Public Hospital gastroenterology day ward and at the Southern Cross Hospital Southern Endoscopy Centre in Christchurch, New Zealand. An amendment to ethical approval was sought and approved in August 2017, which granted the ability to recruit participants from the general population without colonic mucosa biopsy collection. 1,341 people were either invited to be a part of the study or expressed interest in participating; this included 392 people from the general public, 740 people undergoing colonoscopy at Christchurch Public Hospital and 209

people undergoing colonoscopy at Southern Cross Hospital. A total of 349 participants were enrolled before December 21, 2018, 253 from either Christchurch Public Hospital or Southern Cross Hospital, and 96 from the general public (this group did not undergo colonoscopy, but provided all other biological samples). Twenty participants either subsequently declared their unwillingness to participate in the study after initially consenting (15/20) or were withdrawn from the study as a result of findings from colonoscopy (5/20, summarised in Fig. 4). In a study of this size, over a period of this time, not all participants were able to provide all questionnaire data or biological samples as planned. There were a range of reasons for this incomplete dataset including unwillingness to provide some biological samples, lost to follow-up, or technical issues around specimen collection. Therefore, the number of participants who completed each data point and provided biological samples is less than the total number of participants in the study (Fig. 4; Table 1). In addition, colonic biopsies were obviously not available for participants who did not undergo colonoscopy (95). Partici-

Fig. 4. Flow diagram showing overview of the patients recruited for the COMFORT study.

pants were identified as a case (IBS, FC, or FD) or as a control using the Rome IV criteria based on the responses provided by participants in the Modified Hunter New England Survey (MODHNES) questionnaire, 128 participants met the criteria for IBS, 42 met the criteria for FC, and 16 met the criteria for FD (Table 2). There was no significant difference in the diagnostic sub-groups between patients recruited from hospital and the general public ($p = 0.35$; Table 3).

The baseline characteristics of the COMFORT cohort are summarised in Table 2. The mean age of the study participants was 53.4 ± 12.4 years, with a significant difference found between the mean age of those with IBS-M and FC (49.73 ± 13.9 and 57.74 ± 10.5 ; $p = 0.046$); otherwise, the ages of the case groups were not statistically different. However, there was a significant difference between the average ages of the participants recruited in hospital (55.7 ± 10.3) and those from the general public (47.8 ± 1.8 , $p \leq 0.01$, summarised in Table 3). There was a female predominance in the cohort (70% female) and in every sub-phenotype group. The mean economic standard of living among the COMFORT cohort was 35.7 (corresponding to the level “comfortable”) with no significant difference between the sub-phenotype groups ($p = 0.60$). There was no statistically significant difference in education level ($p = 0.31$), alcohol consumption ($p = 0.90$), smoking status ($p = 0.73$ for current smoking and

$p = 0.27$ for having ever smoked), exercise ($p = 0.91$ for walking, $p = 0.68$ for moderate exercise, and $p = 0.27$ for vigorous exercise), or metabolic equivalent to tasks (METs, $p = 0.07$) between the different diagnosis groups. METs were calculated in accordance with the Australian Bureau of Statistics *National Health Survey: Users' Guide* [37]. There were significant differences found between the populations of participants recruited from hospital and those recruited from the general public in having ever smoked ($p \leq 0.01$; Table 3), alcohol consumption ($p = 0.01$), and education ($p = 0.001$). 252 participants were born in New Zealand (80%), 2% reported themselves as being of Māori descent.

Participants who completed the study provided blood, breath, stool, and urine samples where possible; participants were not excluded from the study if they were unable or unwilling to provide the entirety of these samples. Overall 87.5% of stool samples, 90.6% of urine samples, 93.0% of breath samples, 82.4% serum, and 71.4% of PBMC samples were supplied by participants (Table 1); there was no statistically significant difference in the rates of sample collection between the different diagnosis types. However, more serum and PBMC and urine samples were taken from participants recruited from the general public ($p \leq 0.05$). 190/253 (75.1%) of participants undergoing colonoscopy provided colonic mucosal biopsies, this was 60.3% of the total cohort.

Table 1. Numbers and percentages of biological samples collected from participants in the COMFORT study

	IBS				Other FGIDs			Total cases	Control	<i>p</i> value (χ^2)
	IBS-D	IBS-C	IBS-M	total	FC	FD	total			
Participants, <i>n</i>	57	30	41	128	42	16	58	186	129	
Stool, <i>n</i> (%)	53 (93.0%)	25 (83.3%)	35 (85.4%)	113 (88.3%)	38 (90.5%)	15 (93.8%)	53 (91.4%)	166 (89.2%)	121 (93.8%)	0.65
Urine, <i>n</i> (%)	54 (94.7%)	27 (90.0%)	37 (90.2%)	118 (92.2%)	38 (90.5%)	16 (100%)	54 (93.1%)	172 (92.5%)	111 (86.0%)	0.71
Serum, <i>n</i> (%)	48 (84.2%)	25 (83.3%)	33 (80.5%)	106 (82.8%)	34 (81.0%)	13 (81.3%)	47 (81.0%)	153 (82.2%)	99 (76.7%)	0.95
PBMC, <i>n</i> (%)	45 (78.9%)	24 (80.0%)	30 (73.2%)	99 (77.3%)	28 (66.7%)	9 (56.3%)	37 (63.8%)	136 (73.1%)	119 (92.2%)	0.34
Breath, <i>n</i> (%)	56 (98.2%)	30 (100%)	37 (90.2%)	123 (96.1%)	40 (95.2%)	15 (93.8%)	55 (94.8%)	178 (95.7%)	81 (62.8%)	0.33
Tissue, <i>n</i> (%)	38 (66.7%)	17 (56.7%)	26 (63.4%)	81 (63.3%)	27 (64.3%)	9 (56.3%)	36 (62.1%)	117 (62.9%)	73 (56.6%)	0.79

PBMC, peripheral blood mononuclear cell; FGIDs, functional gastrointestinal disorder; FC, functional constipation; FD, functional diarrhoea; IBS-D, irritable bowel syndrome-diarrhoea predominant; IBS-C, irritable bowel syndrome-constipation predominant; IBS-M, irritable bowel syndrome-mixed phenotype predominant.

Table 2. Baseline characteristics of the COMFORT study participants

	IBS				Other FGIDs			Controls
	IBS-D	IBS-C	IBS-M	total	FC	FD	total	
Participants	57	30	41	128	42	16	58	129
Male/female	13/44	2/28	7/34	22/106	12/30	3/13	15/43	57/72
Average age $\pm$ SD, years	52.8 $\pm$ 12	51.1 $\pm$ 14	49.7 $\pm$ 13	51.5 $\pm$ 13	57.7 $\pm$ 10	57.8 $\pm$ 10	57.7 $\pm$ 10	54.4 $\pm$ 16
<i>Education level achieved</i>								
Postgraduate	7 (18.4%)	4 (13.8%)	7 (20.0%)	18 (17.6%)	9 (22.0%)	5 (33.3%)	14 (25.0%)	37 (29.6%)
Tertiary	11 (28.9%)	15 (51.7%)	17 (48.6%)	43 (42.2%)	15 (36.6%)	7 (46.7%)	22 (39.3%)	59 (47.2%)
Secondary	11 (28.9%)	4 (13.8%)	4 (11.4%)	19 (18.6%)	11 (26.8%)	2 (13.3%)	13 (23.2%)	15 (12.0%)
Primary	9 (23.7%)	6 (20.7%)	7 (20.0%)	22 (21.6%)	6 (14.6%)	1 (6.7%)	7 (12.5%)	14 (11.2%)
Currently drink alcohol	44 (80.0%)	23 (74.2%)	33 (80.5%)	100 (78.7%)	34 (81.0%)	12 (85.7%)	46 (82.1%)	102 (79.1%)
Currently smoking	2 (7.7%)	2 (15.4%)	1 (6.3%)	5 (3.9%)	1 (7.1%)	2 (33.3%)	3 (5.4%)	5 (12.2%)
Past smoking	26 (46.4%)	13 (43.3%)	16 (39.0%)	55 (43.3%)	14 (3.3%)	6 (42.9%)	20 (35.7)	41 (31.8%)
<i>Average hours of exercise per week (% participants)</i>								
Walking	366.0 (77.2%)	652.4 (80.0%)	411.7 (74.4%)	452.6 (77.0%)	472.7 (81.0%)	501.4 (50.0%)	477.7 (73.2%)	431.2 (82.0%)
Moderate	225.0 (68.4%)	228.8 (76.7%)	228.8 (77.0%)	227.2 (73.0%)	230.0 (50.0%)	146.7 (64.3%)	205.8 (53.6%)	161.1 (80.0%)
Vigorous	146.8 (30.0%)	97.9 (30.0%)	71.4 (35.9%)	109.4 (31.7%)	94.7 (21.4%)	85.8 (21.4%)	95.3 (21.4%)	96.7 (46.5%)
Average MET	18,620.7	42,114.3	26,733.6	26,725.6	12,545.5	28,294.8	23,950.1	29,586.8

FGIDs, functional gastrointestinal disorders; FC, functional constipation; FD, functional diarrhoea; IBS-D, irritable bowel syndrome-diarrhoea predominant; IBS-C, irritable bowel syndrome-constipation predominant; IBS-M, irritable bowel syndrome-mixed phenotype predominant; MET, metabolic equivalent to tasks.

Discussion

IBS is a major worldwide health problem and is associated with decreased quality of life and significant direct and indirect health care costs [2, 38]. Due to the inherent heterogeneity of IBS, as well as the variety of diagnostic criteria and methodology used, the aetiology and pathophysiology of IBS have remained poorly defined [39]. The COMFORT study was designed to collect, integrate, and

interrogate a variety of pathophysiological data from IBS, FC, and FD cases and compare these to healthy controls to better understand FGID pathogenesis. A systems biology approach will be used to integrate these data in addition to those generated from analysis of the biological samples using a range of platforms. Within the study period, 186 participants met the criteria for FGID cases, and 129 healthy controls. Within the case group, 128 participants met the criteria for IBS (40.6% of the total cohort).

Table 3. Similarities and differences between participants recruited from hospital gastroenterology lists and the general public

	Public	Private	Total	Voluntary	Significance <i>p</i>
<i>N</i>	169	51	220	95	0.3
Female/male	107/62	35/16	142/78	79/16	≤0.01
Average age ± SD, years	56.0±10.4	54.7±9.9	55.7±10.3	47.8±14.8	≤0.01
ELSI-sf living standard score	34.9	36.1	35.2	36.8	0.3
Past smoking, <i>n</i> (%)	76 (45.5)	20 (39.2)	96 (44.0)	20 (21.7)	≤0.01
Currently drinking alcohol, <i>n</i> (%)	136 (81.4)	42 (82.4)	178 (81.7)	70 (74.5)	≤0.01
Education (Bachelor's degree or higher, %)	78 (46)	20 (39.2)	98 (44.5)	56 (59.6)	≤0.01
Average MET	25,849.9	30,548.7	28,199.3	28,544	0.72

ELSI-sf, Economic Standard of Living Index-short form; MET, metabolic equivalent to tasks.

It was found that 80% of the participants were born in New Zealand; however, only 2% of these identified themselves as Māori. In the Canterbury region, 7% of the population identifies as being of Māori descent [40]. The lower population in this study may reflect that Māori populations are more likely to present for colonoscopy with significant “red flag” symptoms [41] and therefore were pre-emptively excluded from participation in this study.

There are few studies of FGIDs in the New Zealand population. As is common in the FGID literature, a range of diagnostic criteria have been used in these studies, making comparison with the COMFORT cohort difficult. Previous New Zealand FGID studies have suggested IBS prevalence of around 20% [42–45]. More recently, a Rome Foundation Working Team reported that the prevalence of IBS in New Zealand (pooled with North America, Europe, and Australia) is 8% (with a 95% confidence interval for 7.0–8.3) [39]. It is likely that differences between countries in the prevalence of FGIDs may reflect cultural differences and variability in health care that lead to reporting differences between countries [39, 46].

The incidence of IBS is greatest in those aged <50 years [47, 48], with the majority of patients first reporting IBS symptoms before 35 years of age [6, 47]. The mean age of IBS participants in the COMFORT cohort was 51.5 years, reflecting the method of recruitment for the COMFORT cohort that focussed on those attending for colonoscopy to obtain a range of biological samples not otherwise available. However, this increased age is consistent with other IBS cohorts recruited internationally which report mean ages closer to 50 years [49, 50]. The Rome Foundation Working Team also reported that the mean age in their large-scale meta-analysis was 40 years [39]. It has been suggested that IBS symptoms can potentially remit over time,

particularly in patients over 50 [6]. However, while abdominal pain has been reported to be milder in patients over 50, they were reported to have a worse quality of life [5].

There was a female predominance in the COMFORT study (70% female overall), which extended to each FGID phenotype in the case group ranging from 93.3 to 71.4%; the control group, while still demonstrating a female predominance, was closer to a 50/50 gender distribution (55.8% female). The female predominance of patients with IBS is well documented [49, 51, 52]. Historically, this was thought to be due to a level of neuroticism in the female patient population [49]; however, it has been demonstrated that there is no difference in health care seeking between male and female patients with IBS [47, 53, 54]. It has also been suggested that female specific sex hormones are associated with the female predominance in IBS symptoms; however, the mechanisms behind this are currently unknown [55, 56]. Genetics (and associated immune activation) are also potential predisposing factors in the pathophysiology of IBS in women [57].

The levels of educational achievement in the COMFORT cohort, of which 86% achieved at least one high school qualification and 46% a bachelor's degree or higher, are higher than that of the New Zealand population in 2015 of 77 and 30%, respectively [58]. However, similarly high education levels were found in an IBS study performed in the United States in 2018, where 28% of the study population had gained a postgraduate qualification [59] compared with 23% in the COMFORT study.

Around 80% of the participants of the COMFORT study had engaged in some form of cardiovascular exercise in the 2 weeks before participation. This is a greater proportion than the general New Zealand population, around half of which (51%) engaged in physical activity

[60]. However, consistent with the findings from the COMFORT cohort, an Australian FGID cohort reported that at least 70% of their IBS participants had performed some exercise in the last 2 weeks [52].

Economic living standards in the COMFORT cohort are higher than that of the New Zealand population with 90% of the COMFORT cohort in the top four (less deprived) ELSI-sf levels “Fairly Comfortable” to “Very Good” compared with 76% in the general New Zealand population [30]; within the COMFORT study, 87% of the cohort fell between the top 3 levels (Comfortable, Good, and Very Good), 12, 40, and 35%, respectively, compared with 61% (22, 29, and 10%, respectively) in the New Zealand population. Furthermore, COMFORT participants are 3 times more likely to score the top ELSI-sf level of “Very Good” (30.5% in the COMFORT cohort compared with 10% in the general population) [30]. There is conflicting evidence about the association of IBS symptoms with socioeconomic status (SES), with evidence existing for association with lower SES [47, 61] and higher SES [62–64], particularly during childhood. However, this observation may also reflect the older age of the COMFORT cohort and also that those who present for health care such as colonoscopy [43], or volunteer for research may be more likely to come from groups with higher living standards [65].

Alcohol consumption among participants in the COMFORT study (79%) was the same as New Zealand national rates for alcohol consumption in people aged 15 years and older [66] and similar to an Australian FGID study in which at least 70% of the cohort drank alcohol [52]. Perceived alcohol intolerance in IBS patients has been reported to be between 12 and 17% [67, 68], and there is some limited mechanistic evidence for the effect of alcohol in worsening IBS symptoms [69]. Mechanistic evidence for the effect of smoking on IBS symptoms, however, is lacking. Having ever smoked cigarettes (defined as greater than 100 cigarettes over a lifetime) was reported by 37.4% COMFORT study participants, and of those who had ever smoked only 11.9% (4.5% of the total cohort) were current smokers at recruitment. This is lower than an Australian FGID study in which a similar proportion of participants had smoked in the past (40%), but 11% reported they still smoke [52]. It is also lower than current New Zealand smoking rates of 16% [70]. Current literature does not support an association between smoking status and IBS symptoms [71, 72].

The baseline demographics of the COMFORT study participants are broadly comparable with other IBS cohorts and the general population. Differences were found between participants recruited from endoscopy lists and

from the general public, where the participants from the general public were younger, smoked, and drank less and a higher proportion of participants had attained postgraduate degrees. As these participants volunteered for the study rather than being invited, these differences were expected as, typically, individuals who volunteer for research are more health conscious than the general public [43, 65]. There was no significant difference between these 2 groups in the diagnosis type, exercise level, ELSI-sf scores, and the female predominance of participants was maintained in both groups. However, future analyses will adjust for possible confounding effects including age, sex, living standard, and smoking status.

Limitations of the dataset collected as part of the COMFORT cohort include the incomplete nature of many of the biological sample datasets. Furthermore, the participants who did not undergo colonoscopy may have had diagnoses other than FGIDs. However, these diagnoses had been made by the general practitioner. Many of the patients will have had a colonoscopy previously or have had non-invasive investigations for other diagnoses (e.g., coeliac disease and inflammatory bowel disease). In the future, subgroup analysis will need to be performed with and without these participants being included.

While the integration of the clinical, dietary, and biological data collected from participants in the COMFORT study has not been presented here, future analyses have been planned. These include the processing of biological samples collected from this cohort (blood, breath, urine, stool, and colonic mucosa tissue) to investigate differences in host-microbiome interactions by measuring faecal composition and predictive functions using shot-gun metagenomics, the faecal, plasma, and urine metabolomes, and host genetics between COMFORT cases and controls. The dietary variables with associated symptoms captured in the FAST diary will be analyzed to determine the role of food groups and specific foods in the genesis of GI symptoms in the COMFORT cohort. Clinical data including medical history and medication use, as well as immediate, current, and chronic GI symptoms, psychological and quality of life data, as well as the baseline demographic data discussed above will be integrated with the results of the biological and dietary data as it becomes available.

In conclusion, the COMFORT cohort is a unique resource that will enable a systems biology approach to be taken to understanding the aetiology and pathogenesis of FGID, particularly IBS, FC, and FD. The cohort is broadly comparable with other IBS cohorts and the general population and will be used independently and collaboratively to further our understanding of FGIDs.

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Statement of Ethics

This study has been conducted in accordance with the protocol, International Conference on Harmonization (ICH) guidelines, applicable national and local requirements, and the ethical principles that have their origin in the Declaration of Helsinki. The COMFORT study protocol was reviewed by the Northern A ethics committee who granted approval (Ref16/NTA/21) as well as the amendment to protocol approved on August 15, 2017. All study participants in the COMFORT study entered the study on a voluntary basis and were able to withdraw from the study at any time with no impact on the standard of their health care. Participant anonymity is respected and protected by password-protected data management systems.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

R.G., N.R., N.T., P.S., K.F., J.C., W.Y., and J.K. conceived and designed the study and analyses. R.G. obtained ethical approval. P.H., S.S., and R.C. collected the data. P.H., R.C., and S.B. performed the analysis. P.H. and R.C. wrote the manuscript. S.S., J.K., S.B., W.Y., J.C., K.F., P.S., N.T., N.R., and R.G. gave critical feedback on the manuscript.

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