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ORIGINAL ARTICLE

Environmental risk factors in inflammatory bowel disease: a population-based case-control study in Asia-Pacific

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ABSTRACT

Objective The rising incidence of inflammatory bowel disease in Asia supports the importance of environmental risk factors in disease aetiology. This prospective population-based case-control study in Asia-Pacific examined risk factors prior to patients developing IBD.

Design 442 incident cases (186 Crohn's disease (CD); 256 UC; 374 Asians) diagnosed between 2011 and 2013 from eight countries in Asia and Australia and 940 controls (frequency-matched by sex, age and geographical location; 789 Asians) completed an environmental factor questionnaire at diagnosis. Unconditional logistic regression models were used to estimate adjusted ORs (aOR) and 95% CIs.

Results In multivariate model, being breast fed >12 months (aOR 0.10; 95% CI 0.04 to 0.30), antibiotic use (aOR 0.19; 0.07 to 0.52), having dogs (aOR 0.54; 0.35 to 0.83), daily tea consumption (aOR 0.62; 0.43 to 0.91) and daily physical activity (aOR 0.58; 0.35 to 0.96) decreased the odds for CD in Asians. In UC, being breast fed >12 months (aOR 0.16; 0.08 to 0.31), antibiotic use (aOR 0.48; 0.27 to 0.87), daily tea (aOR 0.63; 0.46 to 0.86) or coffee consumption (aOR 0.51; 0.36 to 0.72), presence of hot water tap (aOR 0.65; 0.46 to 0.91) and flush toilet in childhood (aOR 0.71; 0.51 to 0.98) were protective for UC development whereas ex-smoking (aOR 2.02; 1.22 to 3.35) increased the risk of UC.

Conclusions This first population-based study of IBD risk factors in Asia-Pacific supports the importance of childhood immunological, hygiene and dietary factors in the development of IBD, suggesting that markers of altered intestinal microbiota may modulate risk of IBD later in life.

Significance of this study

What is already known on this subject?

- The rapid increase in IBD incidence supports the influence of environmental factors.
- Smoking has been consistently shown to be a risk factor for Crohn's disease (CD) and a protective factor for UC.
- Limited epidemiological data suggest a link between having been breast fed and risk of developing IBD.

What are the new findings?

- Breast feeding has a marked protective effect on development of CD and UC; the beneficial effect was most prominent when breast feeding was continued for 12 months or longer.
- A more 'Westernised' diet is a risk factor whereas tea/coffee consumption is a protective factor for IBD.
- Contact with childhood pets is a novel protective factor.
- Inverse association between antibiotic use and development of CD suggests that antibiotics may not be a contributing factor to the rising incidence in Asia.

BACKGROUND

The incidence of IBD has increased dramatically over the past half century.[1] Although more than 160 genetic risk loci have been identified that underlie disease predisposition,[2] these loci have not completely explained the disease aetiology. Striking epidemiological observations including the rising incidence in developing countries and the increased risk of disease in migrant populations implicate the importance of environmental influences on genetic predisposition.[3] In the West, smoking represents one of the most consistently reported risk factors for Crohn's disease (CD),[4] while data are more

Significance of this study

How might it impact on clinical practice in the foreseeable future?

- This is the first prospective study to assess environmental risk factors in a non-Western cohort and a population of emerging disease incidence.
- It identifies early childhood immunological and dietary factors relevant to disease pathogenesis.
- It raises the possibility of disease prevention by modulating early life events in at-risk individuals.
- The strong protective association of breast feeding could potentially motivate female patients with IBD to breast feed, especially since their children have an increased risk of the disease.

conflicting for other factors including appendectomy,[5] tonsillectomy,[6] breast feeding[7–9] and antibiotic use.[3]

The Asia-Pacific Crohn's and Colitis Epidemiology study (ACCESS) is a prospective population-based inception cohort study involving eight countries in Asia and Australia. In the ACCESS study, it was shown that the incidence of IBD varies across Asia but is still lower than in the West.[10] Nonetheless, the emergence of IBD in Asia indicates an important role for environmental factors in the pathogenesis and offers a unique opportunity to study aetiological factors, particularly factors associated with 'Westernisation' including changes in diet, industrial exposure, childhood exposure to antibiotics, vaccination and improved sanitation. Several of these risk factors have not been explored in populations of increasing incidence.

In the current study, we report results of a case-control study examining environmental risk factors prior to the development of IBD in a population-based cohort in Asia-Pacific.

METHODS

Study population

As part of the ACCESS study, we carried out a case-control study across nine countries/regions in Asia-Pacific (China, Hong Kong, Indonesia, Sri Lanka, Macau, Malaysia, Singapore, Thailand and Australia). Full details of subject recruitment have been published elsewhere.[10] In brief, cases comprised of incident IBD subjects diagnosed between April 2011 and March 2013 living in predefined, well-described geographical areas. Study population and centres were selected based on predefined criteria which included well-defined boundaries, geographically isolated and stable population with equivalent access to healthcare, and investigators within the region having the ability to participate in study. Both university and non-university hospitals were included. Details of catchment area, background population and demographics of each region are shown in online supplementary appendix.

Diagnostic criteria, patient clinical demographics and ascertainment methods were standardised. All suspected cases of IBD were referred to gastroenterologists in each hospital and diagnosis of IBD was established on basis of clinical symptoms, endoscopy, histology and radiology. Other diagnoses, including infections, intestinal tuberculosis, amoebiasis and non-steroidal anti-inflammatory drug-induced ulceration, were excluded. Intestinal tissue biopsies were obtained for tuberculosis PCR and culture, and stools were tested for clostridium difficile toxins, microscopy, culture, sensitivity, ova, cysts and parasites. Cases were only included if the diagnosis remained confirmed at 6-month follow-up. At study completion, an audit was performed by external investigators in four randomly selected sites to verify the diagnosis. Ten random case records and medical notes were reviewed to verify diagnosis. Controls were consecutive asymptomatic subjects randomly selected and invited from the streets or departmental stores within the same residential area of cases. Controls were matched on age (±3 years), gender, ethnicity and geographic location. All participants had equal access to healthcare. Family members of IBD cases were not included as controls. Signed informed consent was obtained from all participants. The study was approved by the local Ethics Committees of each centre.

Questionnaire

The questionnaire from the International Organisation of IBD (IOIBD) on environmental factors was used. The questionnaire consists of 87 questions covering 25 different topics proposed to be environmental risk factors for CD and/or UC (see online supplementary appendix – Environmental factors scheme). This questionnaire has been previously used in other studies of IBD cohorts but has not been formally validated.[11–13] Questions relate to five main different areas: (i) Childhood factors up to 20 years including breast feeding, appendectomy, tonsillectomy, eczema, vaccinations (tuberculosis, pertussis, measles, rubella, diphtheria, tetanus, polio), childhood infections (measles, pertussis, rubella, chickenpox, mumps, scarlet fever) and pet ownership; (ii) food habits before diagnosis including daily, weekly or less frequent consumption of fruit, vegetables, egg, cereal, bread, cereal, coffee, tea, juice, sugar and fast food; (iii) smoking habits (current smoker, non-smoker, ex-smoker); (iv) sanitary conditions such as the availability of inhouse water tap, hot water tap or flush toilet; and (v) others factors including daily physical activity, oral contraceptive pill and stressful events before diagnosis. Diet was considered as food habit before diagnosis defined as usual intake over a week. We included an additional question regarding antibiotic use before and after the age of 15 years. Trained investigators/research staff interviewed subjects and completed the questionnaires at inclusion. Total duration of the interview was 20 min for each individual.

Questionnaire translation

Linguistic translation of the IOIBD environmental questionnaire to a Chinese version was formally performed involving two forward and one backward translation by a team of committee members (SCN, WT, JC, YC, SW, CK, HC). Two bilingual translators with proficiency in spoken English and Chinese (SW, CK) independently translated the questionnaire to Chinese. A bilingual gastroenterologist (HC) checked the translations and produced a reconciled translation. Backward translation from Chinese to English language was conducted by a professional translator (YC). The backward translated English version was then verified by comparing it with the original English version by members of the committee. Forward–backward translation was repeated if any major revision was needed. A review meeting was held to solve any discrepancies and one single version of the translation was produced. The Chinese version of the questionnaire was finalised after resolution of any discrepancy and proofreading by the committee. In addition, pilot testing was conducted in five patients and test–retest reliability was assessed in 30 patients. Analyses of test–retest reliability indicated good to strong agreement across all questions (κ coefficient 0.6–1.0). The Chinese version was used in Hong Kong, Macau and mainland China. Similarly, translation of the questionnaire to Sinhala was performed via the same process in Sri Lanka (HJdeS and AK).

Statistical analysis

Data were collected in a web-based database.[10] Subjects in case and control groups were frequency-matched by age (±3 years), sex and location. Assuming the prevalence of the various risk factors among the control group to be in the range of 4%–91%, a sample size of 442 cases and 884 controls was required to detect an OR of at least 2 with a power of 80% at a significance level of 5%. Given that CD and UC share common risk factors, the overall sample size calculation was based on pooled data from IBD as a group. Initial sample size calculation was based on a 1:2 case-control matching. After completion of case recruitment, we added an additional 5% of controls as we anticipate missing data from some controls. The final sample size consisted of 442 cases and 940 controls. Statistical analyses were performed using SPSS statistical software package (SPSS Inc, Chicago, Illinois, USA). Each environmental factor was first tested by univariate analysis with 95% CI. A multi-variate model was then calculated using logistic regression. Initially, a base model was derived including age, sex and socioeconomic status of the countries based on gross national income (GNI) per capita. The economy of each country was divided according to the 2012 GNI per capita, calculated using World Bank Atlas method. Groups were categorised into low income (≤$1035); lower middle income ($1036–$4085); upper middle income ($4086–$12 615); and high income (≥$12 616) (Source: World Bank national accounts and Organisation for Economic Co-operation and Development (OECD) National Accounts data files). Thereafter, each putative predictor for CD and UC was tested adjusting for all of variables in the base model to calculate adjusted ORs (aORs) with 95% CI. Second, any variables with a $p<0.05$ in the univariate analysis were subjected to multivariate analysis. Separate analysis was performed for CD and UC and for Asian patients only. Correction for multiple testing by Bonferroni adjustment was performed.[14] Multivariate logistic regression analysis and Mann–Whitney U test for continuous variables were used. p Value of <0.05 was considered significant.

RESULTS

In total, 442 incident IBD subjects and 940 matched controls answered the questionnaire during the same time period. Of these, 186 (42%) had CD and 256 (58%) had UC. Among the incident IBD subjects, 276 cases were recruited from 2011 to 2012 and 98 cases from 2012 to 2013. The response rate for participation in the questionnaire was 81%. There were more male subjects (58%) with IBD. There was no difference in gender distribution between cases and controls. In all, 84% of the IBD cases were Asians (75% Han Chinese, 15% ethnic Sinhalese, 8% Thai and 2% Indonesian) and 16% were Caucasians (see online supplementary table S1). Median age at recruitment was 38 years (range 25–50) and 39 years (range 26–53) among cases and controls, respectively. IBD patients answered the questionnaire at the point of inclusion which ranged from 0 to 4 weeks from diagnosis. Table 1 showed demographic characteristics of the participants.

Table 1 Demographic characteristics of incident IBD cases and matched controls

Variable	Cases	Controls
Total, n	442	940
Crohn's disease, n (%)	186 (42)	–
UC, n (%)	256 (58)	–
Gender, male, n (%)	258 (58)	517 (55)
Median age, years (range)	38 (25–50)	39 (26–53)
Ethnicity		
Asian, n (%)	374	789
Han Chinese	284 (76)	606 (77)
Indonesian	5 (1)	10 (1)
Thai	30 (8)	60 (8)
Ethnic Sinhalese	55 (15)	113 (14)
Caucasian	68	151

Childhood immunity and infections

In the multi-variate model, several childhood factors were found to be protective for CD and UC. Having being breast fed for 12 months or longer (aOR 0.10; 95% CI 0.04 to 0.30), use of antibiotics before the age of 15 years (aOR 0.19; 95% CI 0.07 to 0.52) and having dogs during childhood (aOR 0.54; 95% CI 0.35 to 0.83) were associated with a reduced risk of developing CD in Asians (table 2). The prevalence of antibiotic use during childhood was 12.5% in Asians and 8.0% in Australians. The reduced risks associated with breast feeding, antibiotic use, having dogs and development of CD were also observed when analysis was performed in the combined Asian and Australian cohorts (table 2). Vaccinations history did not influence the risk of CD when analysis was confined to Asian cohorts. In the combined Asian and Australian cohort, BCG vaccination increased the risk for CD (aOR 2.14; 95% CI 1.03 to 4.42) (table 2).

Having being breast fed for 12 months or longer (aOR 0.16; 95% CI 0.08 to 0.31), antibiotic use in childhood (aOR 0.48; 95% CI 0.27 to 0.87) and having aquarium fish (aOR 0.46, 95% CI 0.29 to 0.73) were protective for the development of UC in Asians. In addition, subjects who had inhouse water tap (aOR 0.67; 95% CI 0.48 to 0.93), hot water tap (aOR 0.65; 95% CI 0.46 to 0.91) or flush toilet (aOR 0.71; 95% CI 0.51 to 0.98) during childhood were less likely to develop UC. When analysis was performed in all incident cases (Asian and Caucasian inclusive), the protective effect of breast feeding, antibiotic use, having fish, and having a hot water tap or flush toilet for development of UC remained statistically significant. Concerning childhood immunisations, vaccination against pertussis (aOR 0.61; 95% CI 0.38 to 0.99) was associated with decreased risk of UC. There were no associations between any of the childhood infections and risk of developing CD or UC (tables 2 and 3).

After adjustment for multiple testing, breast feeding for longer than 12 months remained significant as a protective factor for both CD and UC and having dogs during childhood protected against CD.

Smoking

In Asia, prevalence of smoking was similar between CD cases and controls (11% vs. 11%) whereas in Australia, prevalence of smoking was higher in CD subjects than matched controls (40% vs. 19%). Smoking was not a significant risk factor for Asians with CD. However, smoking was associated with a more than fourfold increased risk of CD when analysis was confined to the Australian Caucasian cohort (aOR 4.30; 95% CI 1.22 to 15.16). In Asians with UC, the prevalence of smoking in cases and controls was similar (11% vs. 11%). More UC patients than controls were ex-smokers in Asia (14% vs. 7%) and Australia (43% vs. 15%). Ex-smoking was associated with an increased

Table 2 Childhood factors and risk of Crohn's disease

	Unadjusted (Asia only)			Adjusted* (Asia only)			Adjusted* (Asia and Australia)		
	OR	95% CI	p Value	OR	95% CI	p Value	OR	95% CI	p Value
Breast feeding§									
0–6 months	1	–	–	1.000	–	–	1	–	–
7–12 months	0.624	0.360 to 1.081	0.093	0.645	0.363 to 1.148	0.136	0.542	0.310 to 0.949	0.032
>12 months	**0.110**	**0.039 to 0.308**	0†	**0.103**	**0.036 to 0.298**	**0†**	**0.086**	**0.030 to 0.243**	0†
Tonsillectomy	0.423	0.055 to 3.245	0.408	0.437	0.056 to 3.400	0.429	1.409	0.568 to 3.500	0.460
Appendectomy	1.035	0.352 to 3.043	0.950	1.021	0.341 to 3.063	0.970	0.675	0.236 to 1.936	0.465
Eczema	1.562	0.915 to 2.665	0.102	1.371	0.786 to 2.392	0.266	1.654	0.968 to 2.827	0.066
Antibiotic use§									
≤15 years	**0.199**	**0.072 to 0.551**	**0.002**	**0.185**	**0.066 to 0.515**	**0.001**	**0.200**	**0.072 to 0.558**	**0.002**
>15 years	0.616	0.334 to 1.137	0.121	0.628	0.339 to 1.162	0.139	0.612	0.331 to 1.132	0.117
Pet animals									
Dog	**0.500**	**0.328 to 0.761**	**0.001**	**0.540**	**0.352 to 0.827**	**0.005**	**0.643**	**0.440 to 0.941**	**0.023**
Cat	0.949	0.602 to 1.496	0.821	1.141	0.714 to 1.824	0.582	1.247	0.819 to 1.898	0.303
Rodents	0.899	0.372 to 2.171	0.812	0.664	0.269 to 1.637	0.373	0.728	0.319 to 1.662	0.452
Birds	0.636	0.311 to 1.301	0.215	0.595	0.289 to 1.225	0.159	0.614	0.327 to 1.153	0.130
Aquarium fish	0.694	0.430 to 1.120	0.134	0.544	0.330 to 0.897	0.017	0.661	0.422 to 1.037	0.071
Vaccinations§									
BCG	1.331	0.616 to 2.876	0.467	0.846	0.375 to 1.906	0.686	**2.136**	**1.033 to 4.418**	**0.041**
Pertussis	1.281	0.650 to 2.521	0.474	0.822	0.401 to 1.683	0.592	0.758	0.387 to 1.488	0.421
Measles	1.779	0.860 to 3.681	0.121	1.021	0.468 to 2.230	0.958	1.413	0.697 to 2.866	0.338
Rubella	1.407	0.786 to 2.520	0.250	0.986	0.519 to 1.875	0.966	0.972	0.528 to 1.790	0.928
Diphtheria	1.476	0.732 to 2.977	0.277	1.045	0.498 to 2.193	0.907	1.125	0.555 to 2.281	0.744
Tetanus	1.352	0.707 to 2.584	0.362	0.907	0.456 to 1.802	0.780	0.998	0.516 to 1.929	0.996
Polio	0.829	0.441 to 1.557	0.559	0.815	0.414 to 1.603	0.552	1.009	0.519 to 1.963	0.979
Childhood infections§									
Measles	0.840	0.444 to 1.591	0.593	1.127	0.582 to 2.183	0.722	1.570	0.895 to 2.755	0.116
Pertussis	1.589	0.333 to 7.579	0.561	2.654	0.537 to 13.120	0.231	2.486	0.505 to 12.248	0.263
Rubella	1.033	0.226 to 4.723	0.967	1.105	0.237 to 5.155	0.898	2.310	0.780 to 6.843	0.131
Chickenpox	0.960	0.638 to 1.445	0.845	0.795	0.512 to 1.234	0.307	0.849	0.558 to 1.290	0.443
Mumps	0.598	0.326 to 1.100	0.098	0.744	0.398 to 1.388	0.352	0.835	0.471 to 1.481	0.538
Scarlet fever	0	0	0.999	0	0	0.999	2.275	0.230 to 22.512	0.482
Sanitary conditions									
Inhouse water tap	1.208	0.803 to 1.819	0.364	0.763	0.477 to 1.222	0.261	0.847	0.533 to 1.344	0.480
Hot water tap	**1.478**	**1.028 to 2.125**	**0.035**	1.000	0.654 to 1.527	0.999	0.940	0.622 to 1.420	0.769
Flush toilet	**1.719**	**1.149 to 2.571**	**0.008**	1.213	0.766 to 1.922	0.411	1.289	0.823 to 2.021	0.267

Statistically significant results are shown in bold (p<0.05).
*Adjusted for sex, age and country income based on GNI.
†Significant at p<0.0006 level after Bonferroni adjustment.
§>20% 'unsure' responses for one of more subject categories.
GNI, gross national income.

risk of UC in Asians (aOR 2.02; 95% CI 1.22 to 3.35) and Australian Caucasians (aOR 3.73; 95% CI 1.14 to 12.16) (tables 4 and 5).

Dietary factors before diagnosis

In univariate analysis, frequent juice intake was associated with increased risk of CD (aOR 1.95; 95% CI 1.02 to 3.72) whereas daily cornflakes intake was associated with an increased risk of UC. Following adjustment, these factors reached borderline significance as risk factors. In multi-variate analysis, daily tea consumption was associated with a reduced risk of CD (aOR 0.62; 95% CI 0.43 to 0.91), whereas both daily tea (aOR 0.63; 95% CI 0.46 to 0.86) and coffee consumption (aOR 0.51; 95% CI 0.36 to 0.72) were associated with a reduced risk of UC in Asians. The protective effect of tea and coffee consumption for IBD development was also observed when analysis was performed in both combined Asian and Australia cohort. Overall, no significant associations were seen between CD or UC and the consumption of fruits, vegetables, eggs, bread, cereal, juice or soft drinks (tables 4 and 5).

Other risk factors

Daily physical activity when compared with less frequent physical activity was found to be protective for CD (aOR 0.58; 95% CI 0.35 to 0.96). There were no significant associations between the use of oral contraceptive pill or cholecystectomy, Westernised diet, or major stressful event before diagnosis and the risk of CD or UC (tables 4 and 5).

Multi-variate model

After including all significant findings from univariate analysis into a multi-variate model, we found that breast feeding ≥12 months, antibiotic use in childhood, early contact with pets, daily tea or coffee consumption and ex-smoking remained significantly associated with IBD development. The only factor that was no longer significant was the association of sanitary

Table 3 Childhood factors and risk of UC

	Unadjusted (Asia only)			Adjusted# (Asia only)			Adjusted# (Asia and Australia)		
	OR	95% CI	p Value	OR	95% CI	p Value	OR	95% CI	p Value
Breast feeding§									
0–6 months	1	–	–	1	–	–	1.000	–	–
7–12 months	1.344	0.906 to 1.994	0.141	1.138	0.758 to 1.709	0.533	1.050	0.708 to 1.557	0.809
>12 months	**0.226**	**0.117 to 0.435**	0†	**0.159**	**0.081 to 0.313**	0†	**0.142**	**0.073 to 0.277**	0†
Tonsillectomy	0.487	0.110 to 2.157	0.343	0.506	0.113 to 2.255	0.371	0.831	0.340 to 2.031	0.831
Appendectomy	0.446	0.133 to 1.498	0.191	0.437	0.129 to 1.474	0.182	0.384	0.135 to 1.092	0.073
Eczema	0.889	0.533 to 1.480	0.650	1.036	0.614 to 1.751	0.893	1.295	0.783 to 2.141	0.315
Antibiotic use§									
≤15 years	**0.441**	**0.245 to 0.791**	**0.006**	**0.484**	**0.267 to 0.874**	**0.016**	**0.503**	**0.279 to 0.907**	**0.022**
>15 years	0.784	0.498 to 1.234	0.293	0.771	0.488 to 1.220	0.267	0.743	0.470 to 1.173	0.203
Pet animals									
Dog	0.846	0.623 to 1.150	0.286	0.818	0.601 to 1.115	0.204	0.796	0.591 to 1.071	0.132
Cat	0.736	0.497 to 1.089	0.125	0.693	0.466 to 1.031	0.070	0.717	0.494 to 1.041	0.080
Rodents	0.978	0.491 to 1.949	0.950	1.162	0.575 to 2.350	0.675	1.187	0.611 to 2.305	0.613
Birds	0.582	0.323 to 1.049	0.072	0.624	0.344 to 1.129	0.119	0.685	0.404 to 1.160	0.160
Aquarium fish	**0.417**	**0.266 to 0.652**	0†	**0.463**	**0.292 to 0.734**	**0.001**	**0.479**	**0.308 to 0.745**	**0.001**
Vaccinations§									
BCG	0.638	0.382 to 1.063	0.085	0.807	0.465 to 1.402	0.448	1.302	0.796 to 2.131	0.293
Pertussis	**0.541**	**0.346 to 0.847**	**0.007**	**0.614**	**0.378 to 0.998**	**0.049**	**0.602**	**0.373 to 0.970**	**0.037**
Measles	0.647	0.411 to 1.018	0.060	0.774	0.472 to 1.271	0.312	0.837	0.527 to 1.328	0.449
Rubella	**0.568**	**0.372 to 0.866**	**0.009**	0.735	0.463 to 1.167	0.192	0.747	0.475 to 1.177	0.208
Diphtheria	**0.588**	**0.370 to 0.937**	**0.025**	0.727	0.440 to 1.201	0.213	0.722	0.442 to 1.181	0.195
Tetanus	**0.572**	**0.364 to 0.900**	**0.016**	0.661	0.405 to 1.078	0.097	0.670	0.436 to 1.144	0.158
Polio	**0.596**	**0.375 to 0.946**	**0.028**	0.681	0.413 to 1.124	0.133	1.012	0.413 to 1.089	0.106
Childhood infections§									
Measles	0.963	0.585 to 1.584	0.880	0.941	0.567 to 1.559	0.812	1.012	0.626 to 1.636	0.962
Pertussis	0.965	0.203 to 4.586	0.965	0.941	0.196 to 4.511	0.939	0.916	0.191 to 4.382	0.912
Rubella	0.314	0.040 to 2.451	0.269	0.353	0.045 to 2.773	0.322	0.318	0.041 to 2.475	0.274
Chickenpox	0.743	0.529 to 1.044	0.087	0.802	0.564 to 1.141	0.219	0.839	0.595 to 1.182	0.316
Mumps	0.845	0.537 to 1.329	0.466	0.822	0.520 to 1.300	0.402	0.806	0.515 to 1.264	0.348
Scarlet fever	**6.247**	**1.036 to 37.680**	**0.046**	5.924	0.979 to 35.845	0.053	3.951	0.789 to 19.778	0.095
Sanitary conditions									
Inhouse water tap	**0.597**	**0.441 to 0.807**	**0.001**	**0.667**	**0.477 to 0.932**	**0.018**	**0.681**	**0.488 to 0.949**	**0.023**
Hot water tap	**0.578**	**0.426 to 0.784**	0†	**0.647**	**0.460 to 0.910**	**0.012**	**0.580**	**0.415 to 0.810**	**0.001**
Flush toilet	**0.618**	**0.461 to 0.830**	**0.001**	**0.707**	**0.508 to 0.983**	**0.039**	0.727	0.525 to 1.008	0.056

Statistically significant results are shown in bold (p<0.05).
*Adjusted for sex, age and country income based on GNI.
†Significant at p<0.0006 level after Bonferroni adjustment.
§>20% 'unsure' responses for one of more subject categories.
GNI, gross national income.

condition including inhouse water tap, hot water tap or flush toilet and the risk of UC (see online supplementary table S2).

Discussion

Our study addressed for the first time, in a large population-based cohort from Asia, the hypothesis that development of IBD is associated with early childhood immunological and hygienic events. We showed that breast feeding was protective for the development of CD and UC. There was a duration-response effect for both diseases whereby protective effect was only significant when duration of breast feeding was greater than 12 months. Childhood contact with pets and consumption of tea and coffee were novel protective factors for disease development in this study. These findings conducted in a large population-based prospectively collected cohort support two emerging hypothesis on IBD aetiology: first, that environmental exposures in early life are implicated in disease aetiology and, second, that markers of altered intestinal microbiota including immunological, hygiene and dietary factors may modulate risk of IBD later in life. Consistent with the literature, ex-smoking was associated with an elevated risk of UC.

The geographical variation of IBD provides an opportunity to investigate possible environmental aetiological factors. The strongest environmental associations identified in the West are cigarette smoking and appendectomy, although neither alone could explain disease variation in Asia. Incidence and prevalence may have stabilised in high-incidence areas such as North America and Europe but they continue to rise in previously low-incidence areas such as Eastern Europe, Asia and much of the developing world.[1,15] This epidemiological shift likely relates to 'Westernisation' of lifestyle, changes in feeding patterns and improved hygiene as part of socioeconomic development in these countries. Environmental influence may occur at different rates in different geographic areas and populations. For instance, southern parts of mainland China including Hong Kong and Singapore are more developed and urbanised than northern

Table 4 Smoking and dietary factors in Crohn's disease

	Unadjusted (Asia only)			Adjusted* (Asia only)			Adjusted* (Asia and Australia)		
	OR	95% CI	p Value	OR	95% CI	p Value	OR	95% CI	p Value
Smoking									
Never	1	–	–	1	–	–	1	–	–
Ex	1.343	0.710 to 2.541	0.365	1.554	0.792 to 3.051	0.200	1.569	0.875 to 2.814	0.130
Current	1.039	0.578 to 1.868	0.897	1.216	0.657 to 2.253	0.534	1.338	0.792 to 2.263	0.277
Fruits (daily)	0.829	0.576 to 1.194	0.315	0.864	0.594 to 1.257	0.445	0.92	0.643 to 1.318	0.650
Vegetables (daily)	0.686	0.434 to 1.086	0.108	0.708	0.444 to 1.131	0.149	0.812	0.512 to 1.289	0.378
Eggs (daily)	1.065	0.707 to 1.603	0.764	0.993	0.655 to 1.505	0.972	0.964	0.645 to 1.440	0.856
Bread§									
>4 day	1.090	0.645 to 1.841	0.749	1.098	0.641 to 1.880	0.734	1.198	0.720 to 1.991	0.487
Wholemeal	1.002	0.639 to 1.573	0.992	1.007	0.637 to 1.590	0.977	1.076	0.706 to 1.642	0.733
Cereal§									
Muesli daily	0.503	0.224 to 1.128	0.095	0.464	0.206 to 1.048	0.065	0.562	0.271 to 1.167	0.122
Cornflakes daily	0.690	0.354 to 1.345	0.276	0.731	0.365 to 1.462	0.376	0.865	0.452 to 1.655	0.662
Juice (≥twice/week)§	**1.952**	**1.024 to 3.721**	**0.042**	1.780	0.918 to 3.451	0.088	**0.443**	**0.249 to 0.788**	**0.006**
Soft drinks (≥twice/week)§	1.756	0.887 to 3.480	0.106	0.742	0.369 to 1.491	0.402	0.759	0.386 to 1.491	0.423
Coffee (daily)§	0.708	0.481 to 1.042	0.080	0.732	0.494 to 1.086	0.121	0.796	0.549 to 1.156	0.231
Tea (daily)§	**0.641**	**0.443 to 0.928**	**0.019**	**0.623**	**0.427 to 0.908**	**0.014**	**0.662**	**0.462 to 0.950**	**0.025**
Western diet (≥50%)									
At present	1.280	0.826 to 1.984	0.270	1.008	0.638 to 1.592	0.974	0.943	0.599 to 1.486	0.800
Before 20-years-old	1.701	1.110 to 2.606	0.015	1.343	0.860 to 2.098	0.194	1.321	0.852 to 2.050	0.214
Physical activities									
Daily	**0.560**	**0.344 to 0.913**	**0.020**	**0.582**	**0.352 to 0.962**	**0.035**	0.655	0.406 to 1.056	0.082
Weekly	0.687	0.445 to 1.061	0.090	**0.543**	**0.345 to 0.854**	**0.008**	**0.639**	**0.416 to 0.982**	**0.041**
Less often	1	–	–	1	–	–	1	–	–
Major stressful event before diagnosis	1.015	0.667 to 1.545	0.946	1.107	0.721 to 1.701	0.642	1.298	0.868 to 1.942	0.204

Statistically significant results are shown in bold (p<0.05).
*Adjusted for sex, age and country income based on GNI.
†Significant at p<0.0006 level after Bonferroni adjustment.
§>20% 'unsure' responses for one of more subject categories.
GNI, gross national income.

regions. There has been conflicting evidence as to whether breast feeding increases the risk or provides a protective role for development of IBD.[9 16 17] Two meta-analyses showed that breast feeding protects against development of CD and UC in adults and early-onset IBD.[7 8] The most striking finding from this study is the marked protective effect of breast feeding (>90%) on the risk of CD and UC; the beneficial effect was most prominent when breast feeding was continued for 12 months or longer. These data are consistent with recent case-control studies from New Zealand and Denmark demonstrating a duration-dependent effect for breast feeding, with a negative association seen after at least 3 and 6 months, respectively.[14 18] In some parts of China, breastfeeding rates at 4 months are 22% compared with the national target of 80%. The potential mechanism of action of breast feeding impacting on development of IBD most likely relates to changes in gut microbiota. Higher concentrations of bifidobacteria and less anaerobic bacteria have been found in stool of breast fed compared with bottle-fed infants.[19] As the gut microbiota continues to change up until 2 years of age, there is a potential need for a longer duration of breast feeding to impact a child's risk of IBD development.

Diet-induced changes to gut-associated microbial communities are suspected to contribute to the growing epidemic of IBD; particularly, increased consumption of refined sugar, fast food, fatty acids, cereals and bread and reduced intake of fruits, vegetables and fibre are associated with disease development.[18 20–22] In the EpiCOM study, Eastern European patients exhibited higher occurrences of 'Westernised' dietary habits than Western European patients.[23] We found that more CD and UC subjects have reported a higher juice and cornflakes intake, respectively when compared with controls. Westernised diet has been shown to induce dysbiosis,[24 25] alter host homeostasis and promote *adherent invasive Escherichia coli* gut colonisation in genetically susceptible mice.[26] In contrast, the consumption of tea and coffee which may contain antioxidants and caffeine was associated with a protective effect. Oral caffeine administration has been shown to ameliorate acute colitis in intestinal epithelial cells.[27] In addition, green tea polyphenols can improve antioxidants levels and attenuated severity of colitis analogous to sulfasalazine.[28]

It has been proposed that a lack of exposure to enteric pathogens in early childhood increases the risk of IBD later in life.[29 30] Although we found no association between childhood infections and disease development, having dogs at a young age markedly reduced risk of CD. Others have shown that exposure to cats before the age of 5 years was protective for later development of CD.[31] It could be that exposure to shed microorganisms from pets at an earlier age modulates the immune system and protects against CD development. In contrast to data from others,[29 30] we found that UC was less common in subjects who had access to a hot water tap and flush toilet during childhood. These data need to be interpreted with caution as many of these factors of modern living may have become ubiquitous since they are now considered normal even in the majority of developing countries.[29]

A striking finding in our cohort, in contrast to the literature, is the protective effect of antibiotics for CD and UC. Several

Table 5 Smoking and dietary factors in UC

	Unadjusted			Adjusted* (Asia only)			Adjusted* (Asia and Australia)		
	OR	95% CI	p Value	OR	95% CI	p Value	OR	95% CI	p Value
Smoking									
Never	1	–	–	1	–	–	1	–	–
Ex	**2.060**	**1.286 to 3.301**	**0.003**	**2.022**	**1.220 to 3.352**	**0.006**	**2.034**	**1.294 to 3.197**	**0.002**
Current	1.159	0.723 to 1.859	0.541	1.091	0.660 to 1.805	0.733	0.912	0.564 to 1.476	0.708
Fruits (daily)	0.869	0.648 to 1.166	0.349	0.875	0.646 to 1.184	0.387	0.952	0.707 to 1.280	0.743
Vegetables (daily)	0.984	0.653 to 1.483	0.940	0.997	0.659 to 1.508	0.987	1.026	0.683 to 1.541	0.903
Eggs (daily)	1.253	0.906 to 1.733	0.173	1.31	0.943 to 1.821	0.107	1.266	0.915 to 1.751	0.155
Bread§									
>4 day	0.881	0.559 to 1.389	0.586	0.779	0.490 to 1.238	0.291	0.856	0.548 to 1.336	0.493
Wholemeal	1.168	0.818 to 1.668	0.393	1.177	0.818 to 1.695	0.380	1.139	0.800 to 1.624	0.470
Cereal§									
Muesli daily	1.317	0.805 to 2.155	0.273	1.463	0.886 to 2.414	0.137	**1.662**	**1.037 to 2.663**	**0.035**
Cornflakes daily	**1.783**	**1.154 to 2.755**	**0.009**	1.483	0.943 to 2.332	0.088	1.496	0.957 to 2.338	0.077
Juice (≥twice/week)§	0.965	0.494 to 1.886	0.917	0.944	0.476 to 1.871	0.869	1.063	0.573 to 1.974	0.845
Soft drinks (≥twice/week)§	1.214	0.640 to 2.300	0.553	1.432	0.742 to 2.762	0.284	1.553	0.831 to 2.899	0.167
Coffee (daily)§	**0.477**	**0.341 to 0.667**	0†	**0.508**	**0.361 to 0.716**	0†	**0.490**	**0.350 to 0.686**	0†
Tea (daily)§	**0.583**	**0.431 to 0.788**	0†	**0.632**	**0.464 to 0.862**	**0.004**	**0.630**	**0.465 to 0.853**	**0.003**
Western diet (≥50%)									
At present	0.681	0.448 to 1.035	0.072	0.77	0.501 to 1.184	0.234	0.723	0.471 to 1.110	0.138
Before 20-years-old	0.868	0.576 to 1.307	0.498	1.004	0.658 to 1.533	0.985	0.984	0.648 to 1.495	0.941
Physical activities									
Daily	0.798	0.549 to 1.161	0.239	0.741	0.507 to 1.084	0.122	0.812	0.562 to 1.175	0.269
Weekly	**0.670**	**0.462 to 0.971**	**0.034**	0.709	0.486 to 1.035	0.075	0.753	0.520 to 1.090	0.133
Less often	1	–	–	1	–	–	1	–	–
Major stressful event before diagnosis	1.009	0.718 to 1.419	0.958	0.984	0.697 to 1.390	0.929	1.098	0.784 to 1.537	0.586

Statistically significant results are shown in bold (p<0.05).
*Adjusted for sex, age and country income based on GNI.
†Significant at p<0.0006 level after Bonferroni adjustment.
§>20% 'unsure' responses for one of more subject categories.
GNI, gross national income.

observational studies have shown an association between antibiotic use and subsequent diagnosis of IBD although causality or biological mechanisms cannot be inferred.[32–34] The paradoxical antibiotic effect could be a surrogate marker of exposure to GI infections and antibiotic use may be markers of frequent childhood infections that lead to induction of tolerance. Our data represent the first to report an inverse association between antibiotic use and development of IBD suggesting that antibiotics may not necessarily be a contributing factor to the rising incidence in Asia. While genetic makeup may protect from IBD, certain environmental factors may also be protective including those related to diet and hygiene such as antibiotic use. Alternatively, an imbalance in normal gut microbiota, due to antibiotic use, might have a sustained effect on GI immune tolerance and sensitivity to pathogens, possibly favouring or protecting the onset of IBD depending on the consequence of changes to the composition of the resident microbiota. This effect could be dependent on the type, duration and time point at which antibiotics were given. It is currently unclear whether the effect of antibiotics on the microbiota is long-lasting, and the importance of transient changes on the microbiota remains questionable. Regarding childhood vaccinations, the association between BCG vaccination and increased risk of CD and UC was particularly convincing, as has been shown by others.[17] BCG is associated with a Th1 immune response.

To date, the most convincing factor shown to be associated with IBD is smoking, with opposite effects on UC and CD.[4] When data were limited to Asian IBD subjects, smoking was not a risk factor for CD. This may be in part because smoking rates in CD was lower in Asia than in Australia. Alternative explanations include differences in genetic makeup and/or environmental exposure (e.g., type of tobacco, way of smoking). It is likely that smoking does not cause CD but modulates the disease once present.[35] Furthermore, smoking in CD may not play the same role in different ethnic groups as it does in Western populations due to differences in genetic heterogeneity.

Our data were consistent with findings from case-control and population-based studies reporting an inverse association between regular physical activity and risk of IBD.[36] In the Nurses' Health study, active women had a 44% reduction in risk of developing CD compared with sedentary women.[36] Although the exact mechanism for this association is not clear, physical activity may induce autophagy and regulate innate immunity to reduce chronic inflammation.[37]

A key strength of this study is that it is one of the first to assess environmental risk factors in a non-Western cohort and a population of emerging disease incidence. Our cohort was unselected and population-based with sizeable samples. All cases were prospectively included and followed-up, hence eliminating the potential bias associated with subjects from referral centres. Both cases and controls were drawn from the same population and geography and were likely to be representative of the general population. The main challenge in investigating environmental risk factors for IBD is that the exposures of interest may occur in early childhood, distant in time from when a patient is diagnosed with IBD. Furthermore, the timing of exposure may be an important predictor of risk. Herein, the collection of data immediately after diagnosis and the short median time from symptom onset to diagnosis of IBD can potentially minimise risk of recall bias and timing of exposures can be more accurately defined. Socioeconomic factors may be responsible for variation in the occurrence of IBD reported worldwide. Given that higher social class has been associated with an increased risk of IBD, we carefully adjusted for socioeconomic status based on GNI for each country. Although our data do not prove causality, they provide evidence suggesting that environmental risk factors for IBD are likely to vary between different populations. Last, we have separated the estimates of CD and UC as the two conditions may have different or opposite risk factors.

Our study results should also be interpreted in the light of several limitations. First and possibly the most important relates to recruitment of controls. An ideal population control would include subjects randomly selected from the electoral roll. However, this was not possible in most centres in Asia. We randomly invited consecutive consenting subjects from the streets within the same residential/postal areas of cases. The use of hospital employees as controls may have potential for bias as such personnel may be more likely to be from a higher socioeconomic stratum. Nonetheless, these individuals comprise the minority of controls.[38] Second, missing data are inevitable in questionnaire studies. Although certain factors including immunisations and infections have had lower response rates, major factors studied which showed significance have received >90% responses. Third, the findings especially questions regarding early lifetime factors are likely to be subjected to recall bias. Compared with hospital-based or retrospective cohort, the inception nature of this study decreases recall bias although elimination of any recall bias is unavoidable in questionnaire-based study. We believe that some factors may be less affected by recall bias than others, for example breast feeding. Wherever possible, data were recorded categorically with 'yes,' 'no' and 'unsure/don't know' to reduce information bias due to participants being forced to provide an answer for questions that they were unsure of. Bias may also be introduced since healthcare professionals interviewed the patients for the questionnaire. Fourth, false positive results may occur due to chance arising from the evaluation of 87 questions. Last, to our knowledge, formal validation of the IOIBD questionnaire has not been conducted.[11] Nonetheless, analyses of test–retest reliability indicated moderate to high reliability across all questions used in the questionnaire. Because questionnaires designed for IBD patients outside of Asia may not be representative of Asian countries, there is a need for a modified questionnaire related to Asian subjects, particularly regarding dietary factors, and for it to be prospectively validated.

In conclusion, we reported for the first time in a population-based cohort in Asia-Pacific the role of dietary and immunological alterations early in life and development of IBD. CD and UC share overlapping environmental factors. We found a duration-response protective association between breast feeding and disease development, and contact with childhood pets is a novel protective factor. These observed associations indicate that early childhood factors and markers of altered intestinal microbiota including antibiotic use may modulate the risk of IBD later in life and that this period requires further evaluation. The strong protective association between breast feeding could potentially motivate female patients with IBD to breast feed, especially since their children have an increased risk of the disease. Attention should be directed from solely investigating the organisms that cause the disease toward factors associated with protection against its occurrence.

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Contributors SCN initiated and designed the study, recruited subjects, collected and analysed the data, and wrote the manuscript. WT and JC performed data entry, data analysis and study audit. RWL, MC, YK, CS, ON, SB, MAK, HJdS, AK, YUS, CJO, K-LL, DO, KLG, IH, QO, Y-FW, PJH, ZHZ, ZRZ, KW, XW, BX, JL, PP, SM, SA, MS, MA, SWCT, TCW, AJH, CMC, HHY, MFL and KKN contributed to the study design, subject recruitment, data analysis and manuscript revision. JCYW, FKLC and JJYS conceived and supervised the study, and revised the manuscript. All authors have seen and approved the manuscript. SCN had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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