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Identification of Environmental Risk Factors Associated With the Development of Inflammatory Bowel Disease

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Abstract

Background and Aims: Multiple genetic and environmental factors are involved in the aetiology of inflammatory bowel disease [IBD] including Crohn's disease [CD] and ulcerative colitis [UC], but data on these exposome factors are difficult to identify. Several exposome factors such as smoking have been shown to be involved; as for other environmental factors, eg stress, results have been conflicting.

Methods: We performed a case-control study including 674 IBD patients of the 1000IBD cohort, frequency-matched based on sex and age with 1348 controls from the population-based Lifelines Cohort Study. Exposome data were obtained using the validated Groningen IBD Environmental Questionnaire [GIEQ], capturing exposome factors through different stages of life using 844 items, of which 454 were applicable to study the role of 93 exposome factors in disease aetiology. Logistic regression [LR] modelling with Bonferroni correction for multiple testing was applied to estimate the multivariable-adjusted effect of each exposome factor.

Results: For IBD, we identified four novel factors: stressful life events (CD odds ratio [OR] 2.61/UC OR 2.92), high perceived stress [2.29/2.67], alcohol use [0.40/0.43], and bronchial hyper-reactivity [3.04/2.36]. Four novel factors were associated with only CD: prenatal smoke exposure [1.89], having a bed partner [0.53], allergies [2.66], and cow's milk hypersensitivity [5.87]; and two solely with UC: carpet flooring [0.57] and neuroticism [1.32]. Nine factors were replicated.

Conclusions: In this study we identified 10 novel, and replicated nine previously reported, exposome factors associated with IBD. Identifying these factors is important for both understanding disease aetiology and future prevention strategies to decrease the development of IBD in genetically susceptible persons.

Key Words: Lifestyle; exposome; environment; aetiology; IBD

1. Introduction

Inflammatory bowel disease [IBD], including Crohn's disease [CD] and ulcerative colitis [UC], has a complexa etiology with a role for the genome, microbiome, and exposome.[1,2]Whereas disease incidence in Western countries has stabilised, incidence rates in Westernizing countries are rising, making IBD a global disease and further emphasising the importance of the so-called exposome.[3] The exposome is a measure of environmental exposures from conception to death, of which some exposures within the Western lifestyle are believed to cause chronic, metabolic inflammation [metaflammation].[4] Identifying the role of the exposome is difficult, but is needed in order to understand the gene-environment interactions and to decrease the incidence of IBD.

Cigarette smoking is probably the best known exposome factor involved in IBD aetiology, with an divergent effect in CD and UC.[5] In the past years however, many studies have examined the role of other exposome factors in IBD aetiology, leading to the identification of a large number of possibly involved factors.[2] One example is formed by the hygiene hypothesis, in which increased hygiene decreases exposure to microorganisms and an subsequent increase of auto-immune disorders, studied through proxies such as living environment [urban versus rural], household pets, and family size during childhood.[6,7] Though important steps in examining the role of the exposome have been made by well-designed previous studies, often questionnaires used are not validated or only a single exposome factor is examined.[8–11] To further increase our knowledge of the exposome and the mechanism of action of these factors, a universal study method is needed.

In this study, we report results of a case-control study in The Netherlands using a validated questionnaire, examining a wide scope of exposome factors in different stages of life prior to diagnosis of IBD.

2. Materials and Methods

2.1. Study population

2.1.1. Cases

All patients from the 1000IBD cohort of the University Medical Center Groningen [UMCG], The Netherlands, were invited to participate in this study. As part of this cohort, patients are prospectively followed while detailed information is collected concerning clinical characteristics as well as extensive phenotype and 'omics' data, described in more detail elsewhere.[12]An overview of the inclusion of participants in this study is shown in Figure 1. Patients were initially recruited for this study through a letter and upon no response, patients were contacted through a phone call or during their visit at the infusion clinic of the UMCG.[13]

2.1.2. Controls

We obtained population-based controls from the Lifelines Cohort Study. Lifelines is a multidisciplinary prospective population-based cohort study examining, in a unique three-generation design, the health and health-related behaviours of 167 729 persons living in the North of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical sociodemographic, behavioural, physical, and psychological factors which contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics.[14] Participants with self-reported IBD or irritable bowel syndrome [IBS] were excluded from this study.

2.2. Questionnaire

The web-based Groningen IBD Environmental Questionnaire [GIEQ] was used to obtain environmental data from patients with IBD.[13] This questionnaire was previously validated by our group, and detailed information about the development of the GIEQ and its validation is published elsewhere.[13] In short, the GIEQ evaluates a wide range of environmental factors, concerning childhood-related exposures [60 items], or exposures during adulthood [361 items], or lifelong exposures [423 items], often split to evaluate time before diagnosis as well as the current situation, giving its users the opportunity to study factors involved in disease aetiology as well as in disease course. For patients without access to a computer, a paper version of the GIEQ was made available [*n*: 82, 11.3%]. Participants in the Lifelines Cohort Study were asked to fill a similar questionnaire upon inclusion.[14] A total of 454 [53.5%] items of the GIEQ, comprising 93 different exposome factors concerning exposures before diagnosis or lifelong exposures, were available for both cases and controls and therefore included in this study.

2.2.1. Data analysis

All participating patients were frequency-matched with controls from the Lifelines Cohort Study in a 1:2 ratio twice: 1] once based on age at diagnosis and gender, to study exposures during childhood and before diagnosis; and 2] once based on age at study inclusion and sex to study personality traits, as these reflect the current situation.

Since all participants with available comparable exposome data within the Lifelines Cohort Study are ≥18 years and older, only patients with an age at diagnosis of ≥16 years were included in this study. Baseline characteristics were compared between participating patients and non-responding patients from the 1000IBD cohort using univariate analysis; for categorical variables, chi square tests were used, and for continuous variables, based on variable distribution, either Mann-Whitney U tests or one-way analysis of variance [ANOVA] tests were used [Supplementary Table 1, available as Supplementary data at *ECCO-JCC* online]. As part of the GIEQ, personality traits are also measured. To examine the role of personality, principal components analysis [PCA] was run on eight sum scores based on the 64-item personality questionnaire within the GIEQ. The suitability of PCA was assessed before analysis. This analysis led to the formation of two components from the Five Factor Model of personality, 'Neuroticism' and 'Conscientiousness', together explaining 66.81% of total variability.[15] Details of the used PCA method and component loadings are in Supplementary Table 2, available as Supplementary data at *ECCO-JCC* online.

Next, all exposures were evaluated using multivariate [MV]-adjusted logistic regression modelling [using the enter method] to estimate the odds ratio [OR] and 95% confidence interval [95% CI] for each independent exposure, while adjusting for the possible confounding effect of gender, age [in years], and smoking status at diagnosis [never/former/current], as a role for cigarette smoking in IBD aetiology was shown repeatedly in past studies.[5] A p-value <0.05 was considered nominally significant. The Bonferroni method, based on the evaluation of 93 exposome factors, was used to determine a significance threshold corrected for multiple testing [p-value $<5.38 \times 10^{-4}$]. To analyse a possible effect modification of gender, all factors statistically significantly associated were analysed

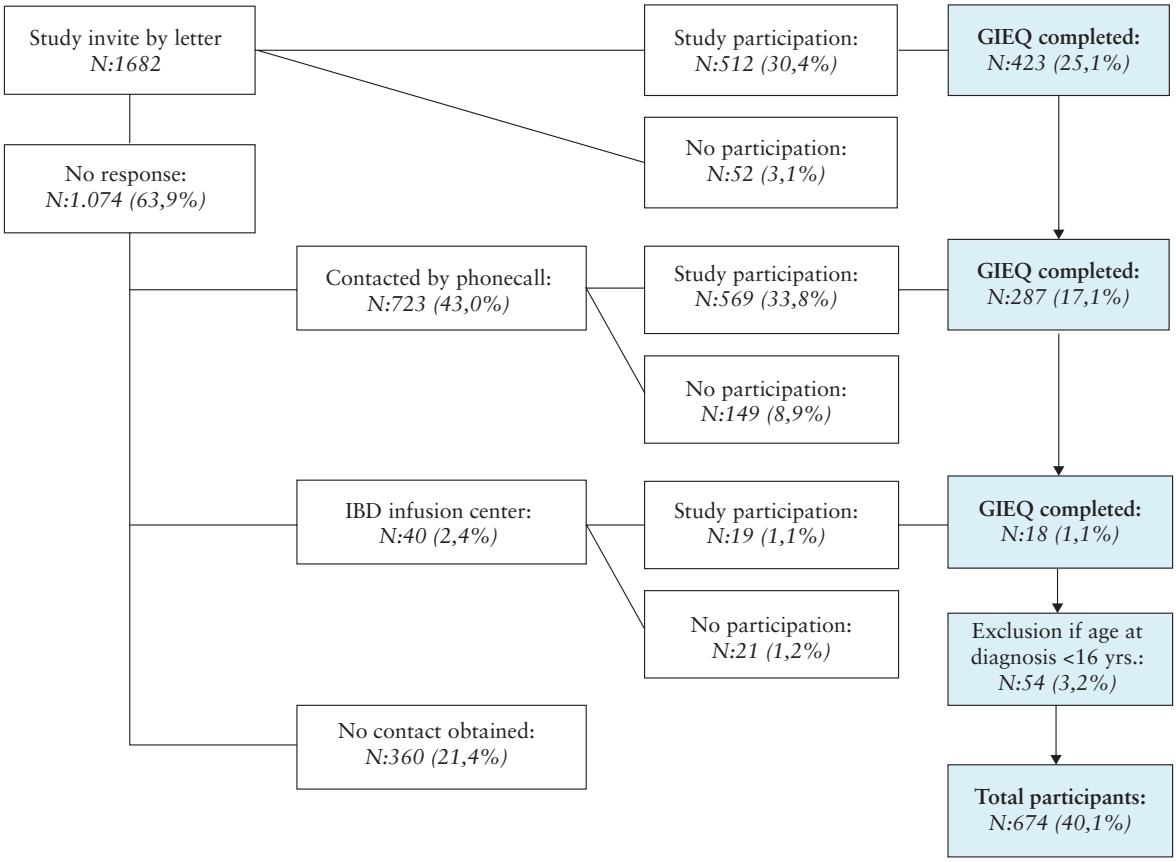

Figure 1. Overview of study inclusion strategies and participation.

Table 1. Baseline characteristics of inflammatory bowel disease [IBD] cases and matched controls.

		Cases	Controls
Total included	*N*	674	1348
Crohn's disease	*N* [%]	323 [47.9]	-
Ulcerative colitis	*N* [%]	321 [47.6]	-
IBD-unclassified	*N* [%]	30 [4.5]	-
Age at diagnosis, in years	Mean [SD]	33.6 [12.9]	33.8 [12.8]
Age at study inclusion, in years	Mean [SD]	50.4 [13.8]	50.3 [13.8]
Gender, female	*N* [%]	415 [61.6]	830 [61.6]

SD, standard deviation.

for risk of IBD stratified by gender [Supplementary Table 3, available as Supplementary data at *ECCO-JCC* online]. Statistical analyses were performed using SPSS statistical software package [SPSS Inc., Chicago, IL, USA].

2.3. Ethical considerations

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in approval by the medical ethical review board of the University Medical Center Groningen, The Netherlands [approval no.: 2017.138], for whom a returned questionnaire was considered as an informed consent.

3. Results

In total, 1682 patients were invited to participate in this study, after which 728 patients [completion rate 40.1%] completed the GIEQ [Figure 1]. In total, 674 patients aged ≥16 years at diagnosis were matched twice, to two sets of 1348 population-based controls, as previously described. Baseline characteristics are described in Table 1. When compared with non-responding patients, participants were more often female and of Western origin, had a higher age and longer disease duration [all p-values <0.05]. There were no baseline differences in educational level [p-value 0.47] nor smoking status [p-value 0.23]; details are shown in Supplementary Table 1.

3.1. Childhood exposures

After examination of 36 childhood-related exposome factors, seven nominally significant associations were found, of which four remained significant after correction for multiple testing [Figure 2]. Prenatal smoke exposure was shown to significantly increase risk of CD [MV-adjusted LR model OR 1.89; 95% CI 1.38–2.59], and nominally significant for UC [OR 1.61; 95% CI 1.16–2.23], as

	Associated factors	CD	UC
Childhood factors	Prenatal smoke exposure		
	Non-western migration		
	Living area first years of life		
	Receiving breastfeeding		
	Household pets age 1		
	Household pets age 1–5		
	Household pets age 5–15		
Adulthood factors	High income		
	High educational level		
	Smoking status		
	Sleeping >8 h per night		
	Stressfull life events		
	High perceived stress score		
	Watching TV >4 h per day		
	Alcohol consumption		
	Red wine		
	White wine		
	Beer		
	Leisure sports		
	Having a roommate/bedpartner		
	Carpet flooring		
	Having a household bird		
Lifelong factors	Appendectomy		
	Tonsillectomy		
	Bronchial hyperreactivity		
	Allergies		
	Cowmilk intolerance		
	Personality neuroticism		

Legend:

Protective association, Bonferroni corrected
Risk increasing association, Bonferroni corrected
Protective association, *P*-value < 0.05
Risk increasing association, *P*-value < 0.05

Figure 2. Heat map of associated exposome factors.

shown in Table 2a. Having siblings was not associated with CD nor with UC [both *p*-values >0.34], but other hygiene markers showed a clear pattern, with an nominally significant increased risk of CD with urban living [OR 1.67; 95% CI 1.19–2.36] during the first years of life. Having household pets during childhood showed a significant protective association, being the strongest effect for pets in the first year of life for CD [OR 0.30; 95% CI 0.22–0.40] as well as UC [OR 0.32; 95% CI 0.24–0.44], but still significant later on in childhood for both CD [OR 0.56; 95% CI 0.42–0.76] and UC [OR 0.47; 95% CI 0.36–0.63], with a comparable effect for different types of pets [Supplementary Table 4]. Birth-related factors showed less association with development of IBD. Whereas a nominal protective effect was seen for receiving breastfeeding in CD alone [OR 0.56; 95% CI 0.37–0.87], no associations were found for birth through caesarian section, preterm birth, birthweight or birth length [all *p*-values >0.15, Supplementary Table 4]. Finally, being born a first-generation non-Western immigrant [birth in a non-Western country] was associated with an increased risk of CD [OR 3.02; 95% CI 1.33–6.38], but not UC [OR 0.58; 95% CI 0.13–2.56], although not significant after correction for multiple testing.

3.2. Adulthood exposures

The role of adulthood exposures was examined through 42 exposome factors. After the initial association of 15 factors, nine remained significant after correction for multiple testing [Figure 2]. A high socioeconomic status was more often seen in controls, with a significant protective association and dose-dependent effect for a high monthly household income in both CD [OR 0.19; 95% CI 0.12–0.29, p_{trend} 4.37 × 10-13] and UC [OR 0.31; 95% CI 0.20–0.49, p_{trend} 1.90 × 10^{-7}], as shown in Table 2b. A moderate educational level was significantly associated with UC alone [OR 0.43; 95% CI 0.29–0.64], but lost significance when analyses were corrected for the potential confounding effect of household income.

Different aspects of adult lifestyle were examined next. A significant risk-increasing association was shown for active cigarette smoking at diagnosis [OR 2.59; 95% CI 1.95–3.44] as well as former smoking [OR 1.51; 95% CI 1.04–2.22], with a dose-dependent effect for the amount of smoked pack-years [p_{trend} 1.01 × 10^{-7}] for CD, whereas only a history of heavy smoking according to pack-years was nominally significant in UC [OR 1.66; 95%CI 1.05–2.61]. A significant protective association with dose-dependent effect was seen for the consumption of alcohol in CD [OR 0.40; 95% CI 0.27–0.60, p_{trend} 5.18 × 10^{-7}] as well as UC [OR 0.43; 95% CI 0.30–0.64, p_{trend} 6.00 × 10^{-6}]. Stratification for smoking status did not alter these findings [data not shown]. When examining alcohol use in more detail, a nominally significant beneficial association was seen for regular consumption of red [OR 0.44; 95% CI 0.25–0.79] and white wine [OR 0.34; 95% CI 0.17–0.68] in CD, but an opposite effect was seen for consumption of beer in UC [OR 1.65; 95% CI 1.12–2.45]. No association was seen for the consumption of other alcoholic beverages nor for the use of different kinds of drugs [all *p*-values >0.05, Supplementary Table 4].

An active lifestyle, as measured by leisure-time sports activities, showed a significant protective effect in CD [OR 0.52; 95% CI 0.40–0.68], with a dose-dependent significant trend for duration of weekly sports activity [p_{trend} 2.58 × 10^{-4}]. In UC, a similar trend was seen [OR 0.74; 95% CI 0.57–0.96], although significance was lost after correction for multiple testing. In contrast, sedentary lifestyle habits, such as a prolonged duration of daily television watching, were nominally associated with a risk increase of UC alone [OR 1.51; 95% CI 1.02–2.24]. Whereas a mean daily sleeping duration of ≥8 h was nominally associated with CD [OR 1.39; 95% CI 1.01–1.92] as well as UC [OR 1.45; 95% CI 1.06–2.00], no effect was seen for sleeping <7 h per night [both *p*-values >0.52]. Stress, as expressed by living through stressful life events [SLE] as well as by a perceived long-term stress score, was shown to be a significant risk for development of IBD. Compared with experiencing 0–1 SLE, having experienced more than three SLE before diagnosis increased the risk of CD and UC almost 3-fold [OR 2.60; 95% CI 1.70–3.99 and OR 2.92; 95% CI 1.92–4.46, respectively]. A similar significant effect was seen for those with a high perceived long-term stress score in CD [OR 2.29; 95% CI 1.53–3.43] and in UC [OR 2.67; 95% CI 1.79–3.98].

Unlike during childhood, hygiene-related factors during adulthood show less effect. In contrast to the strong association described for early-childhood pets, no protective association is seen for pets beforediagnosis [*p*-values ≥0.10], but a nominal risk-increasing effect was seen for having a bird [OR 1.77; 95% CI 1.05–3.01] in CD

Table 2a. Childhood-related environmental risk factors in inflammatory bowel disease.

	Crohn's disease				Ulcerative colitis			
	OR	95% CI		*p*-value	OR	95% CI		*p*-value
Prenatal smoke exposure	1.89	1.38	2.59	8.40×10^{-5}	1.61	1.16	2.23	0.004
Having ≥1 sibling	0.75	0.41	1.37	0.34	0.84	0.45	1.55	0.57
Receiving breastfeeding								
Never	1.00	Ref. group		0.34*	1.00	Ref. group		0.95*
<3 months	0.56	0.37	0.87	0.01	0.84	0.54	1.31	0.45
>3 months	0.84	0.57	1.23	0.36	0.98	0.65	1.48	0.93
Non-Western migration								
Native Dutch	1.00	Ref. group		0.01*	1.00	Ref. group		0.71*
2nd generation migrant	1.20	0.38	3.78	0.76	1.42	0.46	4.38	0.54
1st generation migrant	3.02	1.33	6.83	0.008	0.59	0.13	2.56	0.48
Living area first years of life								
Rural	1.00	Ref. group		0.14*	1.00	Ref. group		0.08*
Large village/small city	0.61	0.44	0.84	0.026	1.08	0.80	1.45	0.61
Urban	1.67	1.19	2.36	0.034	1.42	0.98	2.05	0.06
Household pets								
During first year of life	**0.30**	**0.22**	**0.40**	**4.07×10^{-16}**	**0.32**	**0.24**	**0.44**	**1.03×10^{-13}**
During 15th year of life	**0.37**	**0.29**	**0.49**	**1.20×10^{-12}**	**0.33**	**0.25**	**0.43**	**4.62×10^{-15}**
During 515th year of life	**0.56**	**0.42**	**0.76**	**1.40×10^{-4}**	**0.47**	**0.36**	**0.63**	**2.37×10^{-7}**

All associations significant after Bonferroni correction are shown in bold.
OR, odds ratio; 95% CI, 95% confidence interval.
*p-trend.

but not in UC. Other markers of hygiene did show a significant protective association, such as carpet flooring in UC [OR 0.57; 95% CI 0.43–0.75] and having a roommate/bed partner at time of diagnosis in CD [OR 0.53; 95% CI 0.41–0.70].

Finally, hormonal factors such as age at menarche and the ever use of hormonal contraception, as well as duration of use, showed no association with development of IBD [Supplementary Table 4].

3.3. Lifelong exposures

As not all exposures are bound to a certain stage of life, lifelong exposures possibly involved in IBD were examined as well [Table 2c]. In total, 15 exposures were studied, of which seven showed a significant association [Figure 2]. A history of appendectomy before diagnosis of IBD showed a divergent effect for CD and UC. In CD, a significant risk-increasing association was seen after appendectomy [OR 2.32; 95% CI 1.53–3.51], and a nominally significant 2.70-fold protective association [OR 0.37; 95% CI 0.17–0.81] was shown in UC. A history of tonsillectomy was significantly associated with both risk of CD [OR 2.51; 95% CI 1.91–3.29] and UC [OR 2.05; 95% CI 1.56–2.68]. When only patients with late onset of disease, ≥50 years at diagnosis, were evaluated, a similar effect was seen. CD patients were also significantly more prone to cow's milk intolerance [OR 5.87; 95% CI 2.72–12.68], and all IBD patients experienced more allergy-associated conditions such as pollen hypersensitivity and bronchial hyper-reactivity [all p-values <0.001]. Experiencing multiple allergies was also associated with an increased risk of CD [OR 2.66; 95% CI 1.47–4.80, p_{trend} 8.85×10^{-7}], whereas no clear effect was seen in UC. When allergies were analysed stratified by gender, significance remained only in females [p-value 1.63×10^{-7}], as shown in Supplementary Table 3.

Finally, examining personality traits Neuroticism and Conscientiousness, a high neuroticism score was associated with CD [OR 2.03; 95% CI 1.38–3.02] as well UC [OR 1.84; 95% CI 1.28–2.63] but no effect was seen for conscientiousness [all p-values > 0.57].

4. Discussion

This study shows the importance of environmental risk factors during different stages of life in the development of IBD, in a well-described Dutch cohort of IBD patients and matched population-based controls. In all, 93 factors during different stages of life were systematically evaluated, leading to the identification of 10 novel exposome risk factors as well as the confirmation of nine previously described factors. An overview of all [nominal] significant associations is shown in Figure 3.

To our knowledge, this is the first study describing the risk-increasing association of prenatal smoke exposure in CD especially, while correcting for the potential confounding effect of smoking later in life,[16] although an exact biological pathway underlying this association remains unclear. However, a role for altered DNA methylation patterns, as described in asthma, was hypothesised previously, just as changes in the infant gut microbiota have been described after exposure to prenatal smoke in the general population.[17,18] Further studies are needed to confirm these findings as well as study the effect of early postnatal smoke exposure. Other prenatal- and birth-related factors were not found to be associated with development of CD or UC, in line with previous findings.[19,20] Although a clear protective effect of breastfeeding has been described in the past, this study only showed a nominal significant beneficial effect in CD.[21] The exact reason behind these different findings is unknown; studies from industrialised countries studies suggest strongest effect of breastfeeding in paediatric IBD, whereas the clear dose-dependent effect shown in Asian studies might form an example of the differences of the exposome between East and West.[11,19]

As mentioned previously, the hygiene hypothesis has been described repeatedly in relation to IBD.[2,6] In this study, the protective effect of childhood pets as previously described in a Slovakian study was confirmed.[22]As animal contact is argued to be protective for IBD, due to exposure to harmless micro-organisms, the shown opposite effect for urban living can be viewed within the same line of reasoning and is in line with previous studies. [23,24] This study has also

Table 2b. Adulthood-related environmental risk factors in inflammatory bowel disease.

	Crohn's disease				Ulcerative colitis			
	OR	95% CI		*p*-value	OR	95% CI		*p*-value
Educational level								
Lower level	1.00	Ref. group		0.72*	1.00	Ref. group		0.91*
Moderate level	0.55	0.37	0.81	0.003	**0.43**	**0.29**	**0.64**	**3.40 × 10^{-5}**
High level	0.72	0.48	1.08	0.11	0.71	0.48	1.05	0.08
Monthly income								
Low income	1.00	Ref. group		**4.37 × 10-13***	1.00	Ref. group		**1.90 × 10-7***
Average income	**0.42**	**0.29**	**0.62**	**1.40 × 10^{-5}**	0.63	0.43	0.94	0.024
High income	**0.19**	**0.12**	**0.29**	**4.49 × 10^{-13}**	**0.31**	**0.20**	**0.49**	**3.63 × 10^{-7}**
Smoking status at diagnosis								
Never smoked	1.00	Ref. group		**6.43 × 10-11***	1.00	Ref. group		1.00*
Former smoker	1.51	1.04	2.22	0.032	1.36	0.97	1.92	0.08
Active smoker	**2.59**	**1.95**	**3.44**	**5.55 × 10^{-11}**	0.94	0.68	1.31	0.72
Pack-years of smoking								
Never smoked	1.00	Ref. group		**1.01 × 10-7***	1.00	Ref. group		0.12
Light smoking history	1.49	1.01	2.21	0.047	0.78	0.50	1.22	0.27
Mild smoking history	**2.18**	**1.41**	**3.36**	**4.14 × 10^{-4}**	0.92	0.55	1.52	0.73
Moderate smoking history	**2.46**	**1.63**	**3.72**	**2.10 × 10^{-5}**	1.08	0.68	1.69	0.75
Heavy smoking history	**2.61**	**1.59**	**4.28**	**1.48 × 10^{-4}**	1.66	1.05	2.61	0.03
Alcohol use per day								
<0.65 g	1.00	Ref. group		**5.18 × 10-7***	1.00	Ref. group		**6.00 × 10-6***
0.653.95 g	0.57	0.41	0.81	0.002	0.57	0.40	0.82	0.002
3.9512.63 g	**0.43**	**0.30**	**0.62**	**8.00 × 10^{-6}**	**0.47**	**0.32**	**0.68**	**5.90 × 10^{-5}**
>12.63 g	**0.40**	**0.27**	**0.60**	**5.00 × 10^{-6}**	**0.43**	**0.30**	**0.64**	**2.20 × 10^{-5}**
Type of alcohol often used:								
Beer	1.01	0.67	1.51	0.98	1.65	1.12	2.45	0.012
Red wine	0.44	0.25	0.79	0.006	0.70	0.41	1.20	0.19
White wine	0.34	0.17	0.68	0.003	1.34	0.85	1.12	0.21
Watching television								
≤2 h per day	1.00	Ref. group		0.19*	1.00	Ref. group		0.09*
24 h per day	0.99	0.70	1.41	0.95	0.94	0.65	1.35	0.73
≥4 h per day	1.37	0.92	2.05	0.12	1.51	1.02	2.24	0.039
Sleeping habits								
<7 h versus 7–8 h	1.09	0.73	1.64	0.66	1.13	0.77	1.66	0.53
>8 h versus 7–8 h	1.39	1.07	1.92	0.044	1.51	1.02	2.24	0.039
Having a room-mate/bed partner	**0.53**	**0.41**	**0.70**	**6.00 × 10^{-6}**	0.67	0.51	0.89	0.005
Leisure sports activity	**0.52**	**0.40**	**0.68**	**1.00 × 10^{-6}**	0.74	0.57	0.96	0.025
Duration of sports activity								
Never	1.00	Ref. group		**2.58 × 10-4***	1.00	Ref. group		0.24*
≤1 h per week	0.48	0.30	0.76	0.002	0.71	0.46	1.10	0.13
12 h per week	0.59	0.39	0.89	0.012	0.64	0.41	0.98	0.04
23 h per week	**0.20**	**0.10**	**0.43**	**2.60 × 10^{-5}**	0.52	0.31	0.89	0.017
≥4 h per week	0.63	0.45	0.87	0.006	0.90	0.65	1.24	0.50
Stressful life events								
0–1 life event	1.00	Ref. group		**2.30 × 10-5***	1.00	Ref. group		**7.00 × 10-6***
2–3 life events	1.32	0.98	1.76	0.07	1.25	0.93	1.69	0.14
>3 life events	**2.61**	**1.70**	**3.99**	**1.00 × 10^{-5}**	**2.92**	**1.92**	**4.46**	**6.24 × 10^{-7}**
Perceived long-term stress score								
0–1 points	1.00	Ref. group		0.017*	1.00	Ref. group		0.001*
1.5–3 points	0.84	0.63	1.12	0.22	0.91	0.68	1.21	0.51
>3 points	**2.29**	**1.53**	**3.43**	**6.10 × 10^{-5}**	**2.67**	**1.79**	**3.98**	**1.00 × 10^{-6}**
Use of hormonal contraception	0.75	0.39	1.44	0.39	0.68	0.37	1.28	0.23
Household pets at diagnosis	1.24	0.96	1.61	0.10	0.97	0.75	1.25	0.79
Having a bird as household pet	1.77	1.05	3.01	0.03	0.98	0.52	1.85	0.94
Carpet flooring	0.65	0.50	0.86	0.002	**0.57**	**0.43**	**0.75**	**5.90 × 10^{-5}**

All associations significant after Bonferroni correction are shown in bold.
OR. odds ratio; 95% CI, 95% confidence interval; ref., reference.
**p*-trend.

Table 2c. Lifelong environmental risk factors in inflammatory bowel disease.

	Crohn's disease				Ulcerative colitis			
	OR	95% CI		*p*-value	OR	95% CI		*p*-value
Appendectomy	**2.32**	**1.53**	**3.51**	**7.40×10^{-5}**	0.37	1.17	0.81	0.013
Tonsillectomy	**2.51**	**1.91**	**3.29**	**3.27×10^{-11}**	**2.05**	**1.56**	**2.68**	**1.85×10^{-7}**
Bronchial hyper-reactivity	**3.04**	**2.07**	**4.48**	**1.50×10^{-8}**	**2.36**	**1.60**	**3.50**	**1.70×10^{-5}**
Allergies								
No allergies	**1.00**	**Ref. group**		**8.85 × 10-7***	1.00	Ref. group		0.09*
1 allergy	**1.76**	**1.26**	**2.45**	**0.001**	1.30	0.95	1.79	0.11
2–3 allergies	**2.18**	**1.51**	**3.15**	**3.60×10^{-5}**	1.22	0.82	1.81	0.32
>3 allergies	**2.66**	**1.47**	**4.80**	**0.001**	1.53	0.81	2.90	0.19
Cow's milk intolerance	**5.87**	**2.72**	**12.68**	**7.00×10^{-6}**	1.86	0.76	4.58	0.18
Hayfever	1.09	0.80	1.48	0.58	0.74	0.53	1.02	0.07
Character traits:								
Neuroticism	1.30	1.11	1.51	0.001	**1.32**	**1.14**	**1.54**	**2.09×10^{-4}**
Conscientiousness	1.03	0.88	1.20	0.72	1.00	0.86	1.16	0.99

All associations significant after Bonferroni correction are shown in bold.
OR, odds ratio; 95% CI. 95% confidence interval.
**p*-trend.

identified three novel markers of hygiene during adulthood: with having a bed partner as well as carpeting during adulthood showing a protective association and the opposite is shown for bird ownership. Although it is impossible to adjust for all potential confounders regarding these associations, the potential immune-modulating effect of hygiene during adulthood cannot be ruled out and needs further exploration.

As IBD was first shown in Western countries, a role for socioeconomic status [SES] has been suggested. In line with previous studies, this study has shown an increased risk of CD after immigration from a non-Western country.[25–27] As shown in a previous Swedish study, a high SES was more prevalent in controls.[28] Since the protective association of educational level in this study was likely due to the confounding effect of income status, one might hypothesise that the latter is most important, allowing for healthy lifestyle choices.

Even though 75% of patients themselves indicate stress as a major factor in the development of IBD, past research has mainly focused on stress in regards to risk of relapse.[29] To our knowledge, this is the first study describing the important role of stress and personality in IBD aetiology, whereas previous studies failed to show an association when focused on a specific stressor or only indicated risk-increasing trend.[30,31] As with stress, previous studies examining sleep mostly focus on its role in disease activity.[32] However, one study has shown a U-shaped role for sleep in disease aetiology, implicating a risk-increasing effect for shortened as well as prolonged sleeping duration in UC.[33] Whereas no association for shortened sleeping duration was shown in this study, a nominally significant risk-increasing effect was seen for UC as well as CD for prolonged sleep. Biologically, these findings seem plausible, as an increased sleeping duration as well as stress were previously shown to lead to a pro-inflammatory state, including an 8% increase of C-reactive protein along with a 7% increase of interleukin-6.[34] With the potential risk-increasing effect of a sedentary lifestyle, the opposite effect of physical activity was confirmed in the current study, showing a protective association strongest for CD and nominally significant for UC.[35] Whereas stress and sleep might augment a pro-inflammatory state, regular physical activity was shown to increase autophagy and reduce chronic inflammation. [36]

The role of cigarette smoking might be the best known exposome factor in IBD.[5] Whereas the clear risk-increasing association of smoking in CD was confirmed, in the current study the divergent effect in UC was not shown, possibly due to a lack of power on account of the lowering incidence rates of smoking in IBD populations globally, as well as in our cohort.[37]

Since alcohol is associated with direct mucosal injury and increased bacterial translocation, a risk-increasing effect of alcohol was assumed.[38] However, whereas previous epidemiological studies found no association for alcohol in IBD aetiology, this is the first study describing a potential beneficial effect.[39–41] Although it is possible that cases underestimate their alcohol use or tend to provide 'desired' answers, one would expect the same behaviour in other factors, eg in the evaluation of drug use. Together with average alcohol consumption, this study first described the potential beneficial effect of wine in CD, whereas the opposite is shown for beer in UC.

In contrast to previous findings, our study did not show an association between the use of oral contraceptive pills [OCP] and development of IBD.[42,43] These differences can potentially be explained by the high ever usage of OCP in The Netherlands when compared with the previously studied US cohort [86.7% versus 65.2%], although differences due to different methods used cannot be ruled out.[44]

The previously divergent effect of an appendectomy before diagnosis was confirmed, together with the novel description of a risk-increasing association for a tonsillectomy in both CD and UC.[45,46] As an immune-unbalancing role has been described for the appendix, potentially explaining the protective association for UC after removal, the risk-increasing effect in CD is often attributed to diagnostic bias although a more causal effect cannot be ruled out. [46,47] Contrary to the appendix, the tonsils are thought to have a immune-regulating function which, together with the suggested microbiome composition changes after removal, forms the hypothesised pathway of a risk-increasing association of tonsillectomy in both CD and UC, in line with a recent Danish cohort study.[48–50]

Finally, this study has described strong associations for atopy-related comorbidities in CD as well as UC. Unlike a small Slovakian study previously reported, different types of allergies have been associated with especially CD in our population.[22] Remarkably, self-reported cow's milk intolerance is strongly associated with CD but not UC, in line with a small paediatric study.[51] The effect of

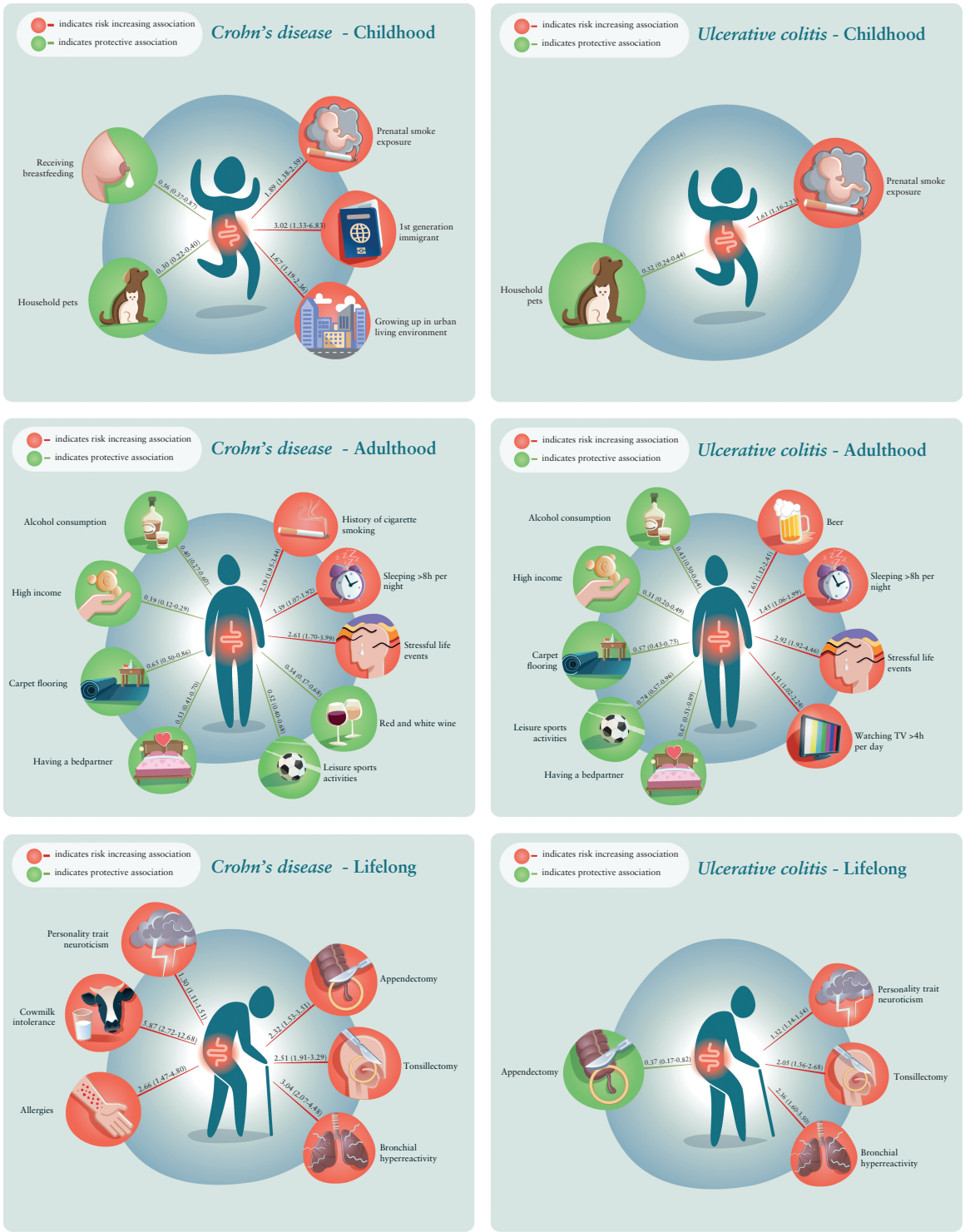

Figure 3. Overview of exposome factors associated with development of Crohn's disease and ulcerative colitis, stratified by stage of life.

diet including milk consumption is currently studied in this cohort. Future studies using universal study methods are needed to validate the described findings. Therefore, the GIEQ is available for use in other IBD cohorts worldwide.

Like all questionnaire-based studies, this study is at risk of recall bias. Although recall bias can never be prevented entirely, several steps were undertaken to limit its effect: 1] due to the smart design of the web-based questionnaire, incorrect answers are limited by the unfolding of follow-up questions only when appropriate based on previous answers; 2] a 'Don't know' option was available for every item to prevent incorrect answering when uncertain; and 3] sections of the GIEQ previously shown to be of low validity were excluded in the current study.[13] As participants were shown to have a longer disease duration than non-participants, the risk of recall bias might be further increased. However, median disease duration of participants was shorter [median 14 years] than

that of the IBD patients within the validation study of the GIEQ [median 19 years].[13]

To our knowledge, this is the first study examining over 90 different exposome factors in association with IBD. The use of the validated GIEQ for this purpose forms a key strength of this study, as previous studies have almost exclusively used invalidated measurement tools.[13] Also, the use of population-based controls from the Lifelines Cohort Study allowed random selection out of 167 729 healthy participants from the same geographical region as cases, limiting the potential bias of differences due to geography-based cultural differences.[14] As patients of the 1000IBD cohort are all treated at a tertiary referral centre, it is possible that more severe phenotypes of disease are over-represented in the current study. However, studying patients enrolled in this cohort also has the great advantage of including only patients with a confirmed IBD diagnosis and strict follow-up by IBD specialists, hindering misclassification due to the use of ICD codes.

Following studies in the field of genetics, future studies focused on the role of the exposome in IBD should be directed towards the use of validated questionnaires, large patient cohorts, and standardised statistical strategies correcting for multiple testing when evaluating a large scope of exposures. These steps would lead to more generalisable results and the opportunity to compare and combine findings between different patient cohorts worldwide.

In this study, we identified 10 novel and replicated nine previously reported exposome factors associated with IBD. Identifying these factors is important for both understanding disease aetiology and decreasing the development of IBD in genetically susceptible persons.

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Conflict of Interest

The authors declare that they have no conflict of interest. RKW has received unrestricted research grants from Takeda, Johnson and Johnson, Tramedico, and Ferring Pharmaceutical Company, has consulted for Takeda Pharmaceuticals, and has received speaker's fees from MSD, Boston Scientific, Abbvie, and Janssen Pharmaceuticals. GD has received unrestricted research grants from Abbvie and Takeda, has joined advisory boards for Mundipharma and Pharmacosmos, and has received speaker's fees from Takeda and Janssen Pharmaceuticals

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Author Contributions

KWJS: study design, data collection, data analysis, writing first draft of manuscript. RKW: collection of clinical data, critical revision of the manuscript. BZA: study design, critical revision of the manuscript. GD: collection of clinical data, study design, critical revision of the manuscript.

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Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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