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The relationship between inflammatory bowel disease and *Helicobacter pylori* across East Asian, European and Mediterranean countries: a meta-analysis

Rabbiaatul Addawiyah Imawana, Daniel Robert Smith, Michaela Louise Goodson

Newcastle University Medicine Malaysia

Abstract

Background The current literature suggests a protective benefit of *Helicobacter pylori* (*H. pylori)* infection against inflammatory bowel disease (IBD). Here we assessed whether this effect varied by IBD subtype—Crohn's disease (CD) or ulcerative colitis (UC)—and geographic region: East Asia, Europe (non-Mediterranean) or Mediterranean region.

Methods A database search was performed up to July 2019 inclusive for all studies that compared *H. pylori* infection in IBD patients vs. non-IBD controls. The relative risk (RR) was used to quantify the association between IBD and *H. pylori*, and the effects were combined across studies using a mixed-effects meta-regression model, which included IBD subtype and geographic region as categorical moderator variables.

Results Our meta-regression model exhibited moderate heterogeneity (I^2=48.74%). Pooled RR depended on both region (P=0.02) and subtype (P<0.001). Pooled RRs were <1 for all subtype and region combinations, indicative of a protective effect of H. pylori against IBD. The pooled RR was 28% (9%, 50%; P=0.001) greater for UC vs. CD and 43% (4%, 96%; P=0.02) greater for Mediterranean countries vs. East Asia. The pooled RR was 18% (-13%, 60%; P=0.48) greater for Europe vs. East Asia and 21% (-13%, 68%; P=0.42) greater for Mediterranean vs. Europe, though these differences were not statistically significant.

Conclusions The protective effect of *H. pylori* on IBD varied by both subtype (more protection against CD vs. UC) and region (East Asia more protected than Mediterranean regions). Variation due to these effects could provide insight into IBD etiology.

Keywords East Asia, Europe, Mediterranean, *Helicobacter pylori*, inflammatory bowel disease

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Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing and remitting inflammation of the gastrointestinal tract, comprising the subtypes Crohn's disease (CD) and ulcerative colitis (UC) [1]. Its global prevalence is estimated to exceed 6 million [2], and in Europe alone more than 2 million people are currently diagnosed with IBD [2-5]. Once considered rare in Asian countries, IBD's incidence has increased considerably in the last 2 decades and prevalence is currently estimated to range between 0.54 and 3.44 per 100,000 persons [1,4,6,7]. IBD has become a disease of global significance, not only compromising quality of life, but also leading to complications such as malnutrition and increased risk of gastrointestinal cancer [5,8-11].

Helicobacter pylori (H. pylori) is well known for its unique ability to colonize the acidic environment of the human stomach [12]. Previous studies have reported that the *H. pylori* infection rate is significantly lower in IBD patients compared to non-IBD controls [13-16]. Moreover, there is the suggestion that this protective effect is stronger for CD vs. UC, and in Eastern vs. Western countries [13-16]. However, studies of "Eastern" populations have been largely confined to East Asia (China, Japan, Korea), whereas studies of "Western" populations have included a broader range of countries from Europe (e.g., Germany, Italy), North

Medical Research Department, Faculty of Medical Sciences, Newcastle University Medicine Malaysia

Conflict of Interest: None

Correspondence to: Rabbiaatul Addawiyah Imawana, Medical Research Department, Faculty of Medical Sciences, Newcastle University, Medicine Malaysia (NUMED Malaysia), No. 1, Jalan Sarjana 1, Kota Ilmu, Malaysia, e-mail: R.A.Binti-Imawana2@newcastle.edu.my

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America (e.g., Mexico) and South America (e.g., Brazil). Such generalized East vs. West contrasts make it challenging to hypothesize about causal agents [13-16], though certain diets, such as the Mediterranean diet, are purported to be protective of IBD [17-19].

Regional and subtype disparities in the protective effect of *H. pylori* on IBD remain circumstantial and have yet to be formally tested in a meta-analytic framework. Such a study is imperative, since significant effects of region and/or subtype may shed light on IBD's etiology, which is currently unclear [20]. This meta-analysis aims to bridge this important research gap by simultaneously incorporating subtype and region in an all-encompassing meta-regression model.

We studied 3 specific geographic regions, namely East Asia, Europe (non-Mediterranean) and the Mediterranean. The selection of these particular regions was guided by existing studies that recognized differences in potential risk factors, IBD characteristics, or features unique to these populations, such as the Mediterranean diet [6,16,17,21-24]. The publication of a sizeable and approximately balanced number of primary studies from East Asian, European and Mediterranean countries is desirable from a statistical power standpoint and provided further motivation for our study. Our meta-analysis aimed to address the following research questions:

1. Does the association between IBD and *H. pylori* vary by IBD subtype?
2. Does the association between IBD and *H. pylori* vary by region?
3. Is there an interaction effect of IBD subtype and region on the association between IBD and *H. pylori*?

Materials and methods

This meta-analysis followed the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [25].

Inclusion and exclusion criteria

Inclusion criteria for studies in our meta-analysis were: 1) studies that examined the association between *H. pylori* and IBD; 2) studies that included adult populations; 3) studies conducted on populations from European, East Asian or Mediterranean countries; 4) studies that reported exact numbers of IBD patients for CD and/or UC subtypes; and 5) studies either originally available in English or could be translated into English. Studies were excluded when they: 1) focused on pediatric populations; 2) focused on countries or regions other than those specified in the above inclusion criteria; and 3) lacked a control group required to compute the effect size (relative risk [RR], see below) to quantify the association between *H. pylori* and IBD.

Search strategy

A rigorous database search was performed using Scopus, Ovid MEDLINE, Embase, PubMed and Cochrane Library for studies published up until June 2019 inclusive. Our search strategy included Medical Subject Heading (MeSH) terms and keyword combinations such as "Inflammatory Bowel Disease", "IBD", "Ulcerative Colitis", "UC", "Crohn's Disease", "CD", "*H. pylori*" and "*Helicobacter pylori*". Boolean search operators "AND" and "OR" were used to combine search terms. We also performed manual searches using references from studies retrieved for any additional relevant studies.

Data extraction

Information retrieved from selected studies included titles, authors, publication year, study design (cohort or case-control), population age group, population origin or region of the studies, *H. pylori* detection method, and sample sizes in *H. pylori* (positive/negative) and IBD (case/control) groups. IBD patients were further divided into CD and UC subtypes, along with their results for *H. pylori* infection. Studies were separated into 3 groups, namely East Asian, European (excluding Mediterranean) and Mediterranean, according to the geographic region in which they were conducted.

Risk of bias

The Newcastle-Ottawa scale was used to assess the risk of bias in individual studies [26,27]. Studies were first grouped based on their design, such as case-control or cohort studies, before being assessed for bias. Studies with a score of ≥7 were regarded to be of "higher quality", implying a lower risk of bias [27].

Statistical analysis

Meta-analysis was performed using R (predominantly using the metafor and ggplot2 packages), formally known as The R Project for Statistical Computing version 3.6.1 R Core Team (2019) [28-30] (see supplementary material for full reproducible R code and master dataset). From each primary study, we extracted the counts of positive and negative cases of *H. pylori* in the IBD and non-IBD groups. In line with previous meta-analyses that combined cohort and case-control studies [13-16], we used the RR as our effect size metric to quantify the magnitude of the association between IBD and *H. pylori* incidence. We fitted a hierarchical weighted mixed-effects meta-regression model [31] of the log RR, which included region (levels: E. Asia; Europe; Mediterranean) and subtype (levels: CD; UC) as categorical (dummy-coded) moderator variables, with the amount of residual heterogeneity estimated using maximum likelihood. To our knowledge, none of our included studies shared the same dataset. However, 19 studies included data on both UC and CD, implying correlated sampling errors.

Accordingly, we included the study name and observation id as random effects (where observation id was nested in study name) to account for non-independence at the study level. To test the significance of a possible region × subtype interaction, we performed a likelihood ratio test, comparing the full model (containing region and subtype main effects and the region × subtype interaction term) with a reduced model containing only the additive main effects of region and subtype. We present model predictions (i.e., weighted averages or summary effects) as RRs and corresponding confidence intervals for all moderator combinations. We did not present an overall effect since this is somewhat meaningless and often misleading in the presence of moderators [32]. Contrasts between levels of subtype and region variables (e.g., RR Mediterranean vs. RR E. Asia) were expressed as ratios of RR (RRR) with confidence intervals and P values adjusted for simultaneous inference using the single-step method. Statistical heterogeneity was assessed using Cochran's Q test (threshold P-value <0.10) and Higgins test (I^2) (low heterogeneity: I^2<25%; moderate heterogeneity: I^2 25-75%; high heterogeneity: I^2>75%) [33,34]. We performed leave-one-out sensitivity analysis, where the model was iteratively re-fit after omitting each respective study to examine the effect on predictions (RR) and contrasts (RRR). The pseudo Egger regression test was used to assess for small study bias. Here, study variances were included in the selected meta-analysis model as an additional moderator and a P-value was computed to test the null hypothesis that the intercept term was equal to zero (rejection of the null implied evidence of small-study bias). The standard funnel plot was also used to assess for small-study bias and the contour-enhanced funnel plot was used to assess whether any such bias might be attributed to publication bias. With the exception of Cochran's Q test, we set our significance threshold at α=0.05 (i.e., 5%), accordingly computing 95% confidence intervals.

Results

Search results and main characteristics of studies

A total of 477 relevant studies were identified through database and manual searches. After screening of the titles and abstracts, 418 irrelevant studies were excluded. The remaining 59 articles were retrieved for detailed evaluation. Upon further inspection, 32 articles were included in the final meta-analysis, while 27 studies were excluded on the following grounds: 3 were focused on pediatric populations; 4 were unavailable in full text; 2 were unavailable in English; 11 were conducted on populations outside our regions of interest; 6 did not have a non-IBD control group; and 1 study was unclear in the results pertaining to IBD subtypes. A detailed flow diagram of our study selection process is shown in Fig. 1. The main characteristics of the included studies are listed in Table 1. They include all 3 regions of interest: East Asia, Europe and the Mediterranean [22,35-65]. In our meta-analysis, 22 studies were case-control studies, while 10 were cohort studies. Of the 32 studies, 17 of them (53.12%) scored ≥7 on the Newcastle-Ottawa scale. In total, the 32 studies in this meta-analysis included 4607 IBD cases and 4666 controls.

Effect size estimates (RR) and contrasts (RRR)

In this meta-analysis, 24.33% of patients with IBD had *H. pylori* infection, compared to 43.12% in the non-IBD control group. A comparison of the full and reduced meta-regression models showed no evidence of a significant subtype × region interaction (likelihood ratio test: λ_{LR} =1.07; P=0.59) on RR. We therefore used the reduced version as the main model in this study. Sensitivity analysis showed that the predictions (RR) and contrasts (RRR) from this model were generally robust in response to the omission of any particular study (see supplementary material). This model exhibited moderate heterogeneity (I^2=48.74 %; Cochran's Q test: χ^2_{49} =100.55, P<0.001). Both region (Wald test: χ^2_2 =7.73, P=0.02) and subtype (Wald test: χ^2_1 =13.98, P<0.001) were both statistically significant as main effects (omnibus Wald test of both moderators χ^2_3 =21.87, P<0.001).

Pooled RR's were <1 for all subtype and region combinations, implying a negative association between *H. pylori* and IBD. Model predictions (i.e., summary effects presented as RR with corresponding lower and upper 95% confidence bounds) were as follows: CD-East Asia 0.43 (0.36-0.52); CD-Europe 0.51 (0.42-0.61); CD-Mediterranean 0.62 (0.50-0.76); UC-East Asia 0.55 (0.46-0.66); UC-Europe 0.65 (0.54-0.78); and UC-Mediterranean 0.79 (0.64-0.96) (Fig. 2). The protective effect of *H. pylori* appears to be greatest in East Asian regions (lowest RR), followed by European regions, while Mediterranean regions have the least protective effect in both CD and UC subtypes. In addition, the protective effect seems to be greater for CD than for UC across all regions. Although the pooled RRs were <1 for all subtype and region combinations, the RR was 28% (9%, 50%) greater for UC vs. CD (RRR=1.28 [1.09, 1.50], P<0.001) and 43% [4%, 96%] greater for Mediterranean vs. East Asia (RRR=1.43 [1.04, 1.96], P=0.02) (Fig. 3). Pooled RRs were 18% (-13%, 60%) greater for Europe vs. East Asia (RRR=1.18 [0.87; 1.60], P=0.48) and 21% [-13%, 68%] greater for Mediterranean vs. Europe [RRR=1.21 [0.87; 1.68], P=0.42), though these differences were not statistically significant.(Fig. 3).

Publication bias

A funnel plot showed slight asymmetry (Fig. 4), suggestive of possible small-study bias, also suggested by the pseudo Egger regression test: (Z=-6.35; P<0.001). However, the contour-enhanced funnel plot shows approximate symmetry and suggests that publication bias is unlikely (Fig. 5).

Discussion

This is the fifth meta-analysis studying the relationship between *H. pylori* and IBD [13-16]. Our meta-analysis has

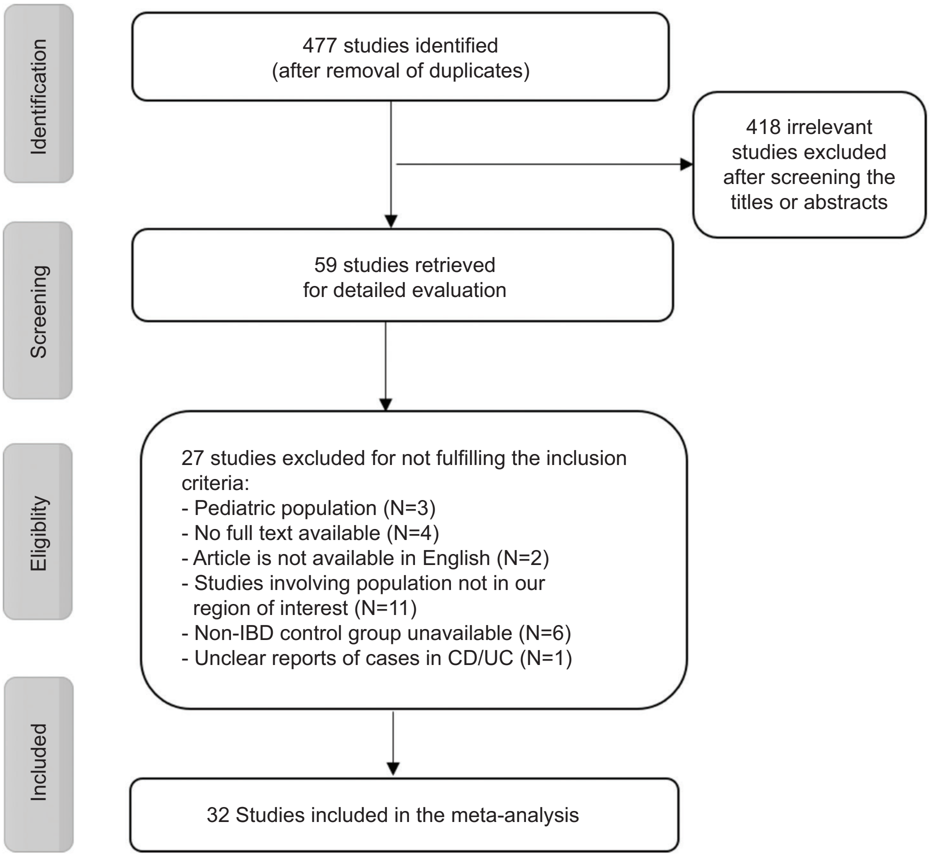

Figure 1 Study flow diagram showing identification, screening and eligibility stages which resulted in 32 studies being included in our meta-analysis
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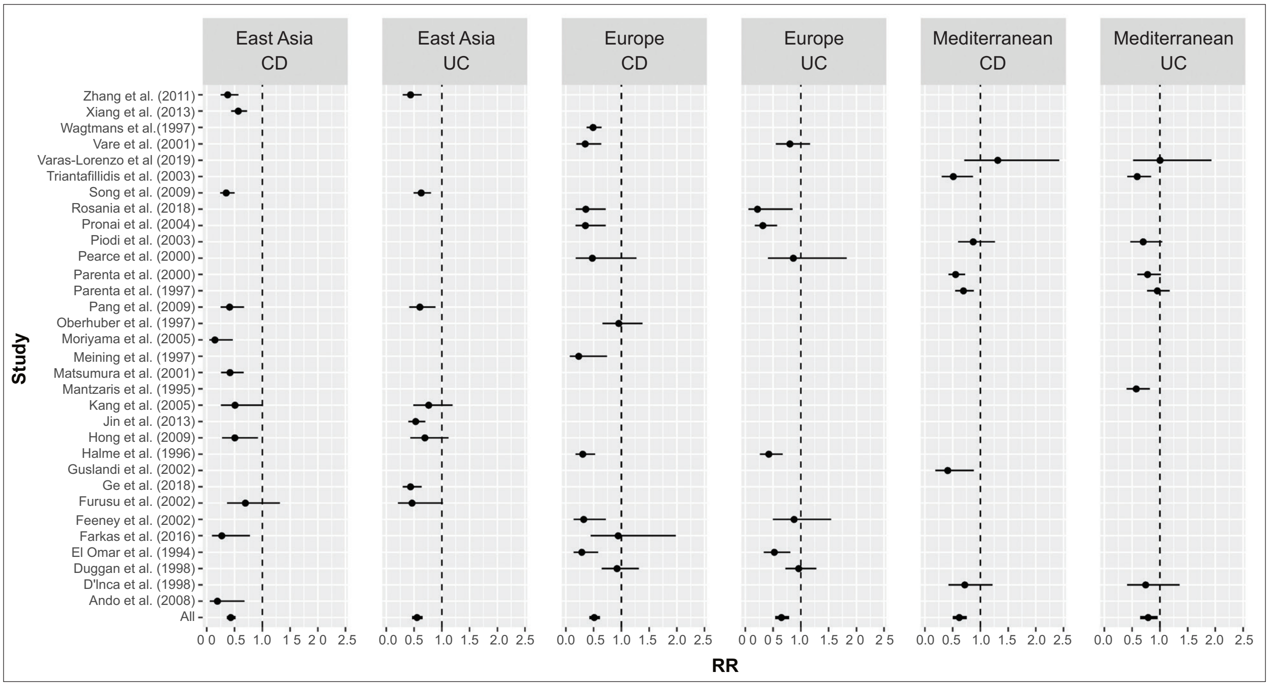

Figure 2 Relative risks (RR) and 95% confidence intervals for each primary study by region and subtype (CD = Crohn's disease; UC = ulcerative colitis. The pooled summary effects are shown at the base of the plot aligned with 'All'. Vertical dashed lines are shown at RR=1 to indicate the null effect (confidence intervals that do not include 1 are statistically significant at P<0.05)

Table 1 Characteristics of studies included in the meta-analysis

Study [Ref.]	Year	Population	Study design	Risk of bias (NOS)	IBD patients CD/UC (n)	Controls (n)	Total (n)	Control group source	*H. pylori* diagnosis
Matsumura *et al* [35]	2001	Japan	Cohort	6	90/NA	525	615	Healthy control	Serology
Furusu *et al* [36]	2002	Japan	Case Control	6	25/25	25	75	Non-IBD Patients	Serology/Histology
Moriyama *et al* [37]	2005	Japan	Case Control	6	29/NA	7	36	Non-IBD Patients	UBT
Ando *et al* [38]	2008	Japan	Cohort	7	38/NA	12	50	Healthy Control	UBT
Kang *et al* [39]	2006	South Korea	Case Control	6	21/28	151	200	Non-IBD Patients	Histology
Song *et al* [40]	2009	South Korea	Case Control	7	147/169	316	632	Healthy Control	UBT
Hong *et al* [41]	2009	South Korea	Case Control	5	37/43	41	121	Non-IBD Patients	Histology
Pang *et al* [42]	2009	China	Case Control	8	52/54	106	212	Healthy Control	Serology
Zhang *et al* [43]	2011	China	Case Control	7	104/104	416	624	Healthy Control	UBT
Jin *et al* [44]	2013	China	Case Control	6	NA/153	121	274	Non-IBD patients	UBT/Biopsy sample culture
Xiang *et al* [45]	2013	China	Case Control	5	229/NA	248	477	Non-IBD patients	UBT/Biopsy sample culture
Ge *et al* [46]	2018	China	Case Control	6	NA/146	150	296	Healthy Control	RUT/Histology
Parente *et al* [47]	1997	Italy	Cohort	7	123/93	216	432	Blood donors	Serology
D'Inca *et al* [48]	1998	Italy	Cohort	6	67/41	43	151	Non IBD Patients	Histology
Parente *et al* [49]	2000	Italy	Cohort	7	141/79	141	361	Non IBD Patients	UBT/Histology
Guslandi *et al* [50]	2002	Italy	Case Control	3	60/NA	30	90	Irritable Bowel Syndrome Patients	Serology
Piodi *et al* [51]	2003	Italy	Case Control	8	30/42	72	144	Non IBD Patients	UBT
Mantzaris *et al* [52]	1995	Greece	Case Control	7	NA/90	120	210	Healthy Control	Serology/RUT
Triantafillidis *et al* [53]	2003	Greece	Case Control	7	39/77	127	243	Healthy Control	Serology
Varas-Lorenzo *et al* [54]	2019	Spain	Case Control	6	40/40	20	100	Non-IBD Patients	UBT
El-Omar *et al* [55]	1994	United Kingdom	Case Control	7	63/47	100	210	Non IBD Patients	Serology
Duggan *et al* [56]	1998	United Kingdom	Case Control	7	110/213	337	660	Non IBD Patients	Serology
Pearce *et al* [57]	2000	United Kingdom	Cohort	8	42/51	40	133	Irritable Bowel Syndrome Patients	Serology, UBT
Feeney *et al* [58]	2002	United Kingdom	Case Control	6	139/137	276	552	Non IBD Patients	Serology
Halme *et al* [59]	1996	Finland	Case Control	5	100/100	100	300	Non IBD Patients	Serology

(Contd...)

Table 1 *Continued*

Study [Ref.]	Year	Population	Study design	Risk of bias (NOS)	IBD patients CD/UC (n)	Controls (n)	Total (n)	Control group source	*H. pylori* diagnosis
Vare *et al* [60]	2001	Finland	Cohort	7	94/185	70	347	Healthy Control	Serology
Wagtmans *et al* [61]	1997	Netherlands	Case Control	8	386/NA	277	663	Blood donors	Serology
Meining *et al* [62]	1997	Germany	Case Control	7	36/NA	36	72	Healthy Control	Histology
Oberhuber *et al* [63]	1997	Germany	Cohort	6	75/NA	200	275	Non IBD Patients	Histology
Rosania *et al* [64]	2018	Germany	Case Control	9	90/37	257	384	Blood Donors	Serology
Pronai *et al* [65]	2004	Hungary	Cohort	6	51/82	200	333	Non IBD, COPD Patients	UBT
Farkas *et al* [22]	2016	Hungary and Hong Kong	Cohort	7	180/NA	189	369	Non IBD Patients	Histology

H. pylori, Helicobacter pylori; IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; NOS, Newcastle-Ottawa Scale; NA, not applicable; UBT, urea breath test; RUT, rapid urease test; COPD, chronic obstructive pulmonary disease

extended previous work by simultaneously quantifying the impact of geographic region (East Asia, non-Mediterranean Europe, Mediterranean) and IBD subtype (CD, UC) on the association between *H. pylori* and IBD. We found significant effects of both region and IBD subtype, though there was no evidence to suggest these effects were interactive, i.e., that the difference between IBD subtypes varied across regions, or *vice versa*. Although the RR for all region and subtype combinations suggest that *H. pylori* infection has a protective effect against the development of IBD, the RR was 28% greater for UC than CD (pooled across all regions), and 43% greater for Mediterranean compared to East Asian regions (pooled across subtypes).

The protective effect of *H. pylori* infection on IBD incidence has strong support [40-43,48,53,58,60,65], despite some studies suggesting that a lower *H. pylori* infection rate may be an artefact of IBD treatment (e.g., sulfasalazine, mesalazine, corticosteroids, antibiotics, etc.) eradicating *H. pylori* in some patients [47,51,52]. IBD is known to initiate an increase in type 1 T helper lymphocyte (Th1) and/or T helper 17 cells (Th17), resulting in higher inflammatory factors [66]. Moreover, CD patients have a tendency for selective activation of Th1- and Th17-related cytokines, which possibly explains the greater protective effect of *H. pylori* in CD compared to UC [16]. This trend was suggested by previous meta-analyses [13-16], although we are the first to confirm the effect using formal hypothesis tests.

Previous meta-analyses have suggested that the beneficial effects of *H. pylori* on the risk of IBD are greater for eastern than for western populations [16]. A possible explanation is the greater relative abundance of the seropositive CagA *H. pylori* strain in East Asian compared to western populations [23]. It has been suggested that expression of CagA might increase the production of beta-defensins, thought to play a protective role in IBD pathogenesis [23]. Another possible theory is that during *H. pylori* infection, the response of the type 2 T helper cell 2 (Th2) cytokine is dependent on the presence of CagA strain [23]. Indeed, this specific response has been shown to be protective against gut inflammation [23].

Genetic disparities in the patients themselves might also explain the variation in the protective effect of *H. pylori* across geographic regions. A possible hypothesis is that IBD in East Asian populations is less likely to be attributed to genetic factors compared to those in the west. In Asian populations, a family history of IBD is not frequently observed, relative to counterparts in Europe or North America [24,67]. One of the most studied genes in IBD heritability in the West is the *nucleotide-binding oligomerization domain-containing protein 2* (*NOD2*) [67,68]. Genetic studies have found no evidence of a correlation between *NOD2* and IBD in Asian populations [69-71]. However, new *NOD2* mutations such as (*JW1*) have been reported in Malaysian populations, while increased expression of (*P268S*) was reported in Han Chinese and Indian populations [69-71]. In a genome-wide association study (GWAS) that investigated the role of single nucleotide polymorphism (SNP), the *autophagy-related 16-like 1* gene (*ATG16L1*) and *immunity-related GTPase family M* gene (*IRGM*) were found to potentially increase susceptibility

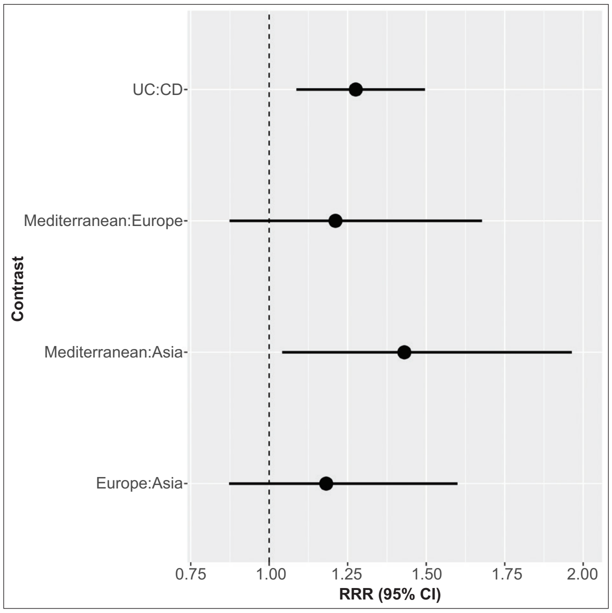

Figure 3 Ratios of relative risks (RRR) and 95% confidence intervals for pre-specified contrasts of moderator variables (region and subtype) included in our meta-regression model. RRR represent average effects, pooled over levels of the other moderator in the model. Confidence intervals are adjusted for simultaneous inference using the single-step method. The vertical dashed line at RRR=1 indicates the null effect (confidence intervals that do not include 1 are statistically significant at P<0.05)

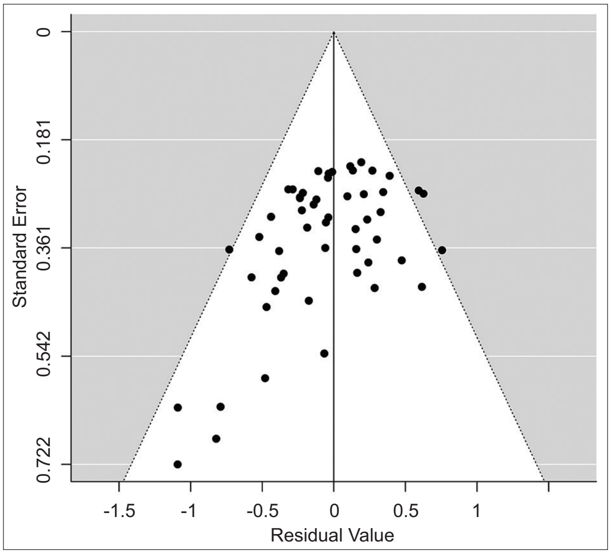

Figure 4 Standard funnel plot for all studies included in our meta-regression model. The cluster of studies on the lower left side might be suggestive of small study bias

for IBD in Western populations [72]. Similar studies on Asian populations have shown mixed results in Korea and Japan [67,72]. Currently, more than 230 SNPs have been linked to IBD through GWAS, though their exact roles and mechanism of action in IBD pathogenesis are yet to be fully described [67,73].

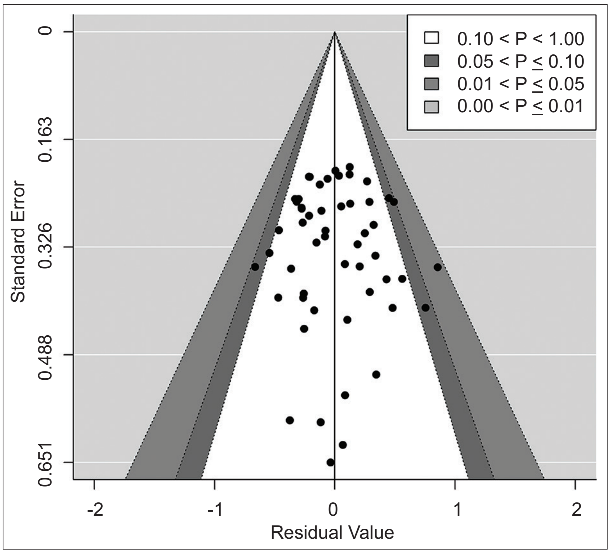

Figure 5 Contour-enhanced funnel plot for all studies included in our meta-regression model. The plot is approximately symmetrical and is not indicative of publication bias. All of the smaller studies (larger standard error towards base of plot) lie within 0.10<P<1.00

Regional variation in the protective effect of *H. pylori* on IBD might also be ascribed to environmental and socioeconomic factors, such as the role of diet. The western diet is thought to be particularly conducive to IBD, owing to the low intake of fiber and high intake of refined carbohydrate and processed meat [18,74]. Indeed, as IBD incidence continues to rise in Asia [7], many studies have suggested that this could be the result of Asian populations adopting the western diet [18,74-76]. The Mediterranean diet, plant-based diet and semi-vegetarian diet have been shown by some studies to help alleviate symptoms of IBD and keep IBD patients in remission [17,77,78]. It is noteworthy that the Mediterranean diet has also been reported to reduce inflammation and improve microbiota in IBD patients [17-19]. Interestingly, our meta-analysis found that the protective effect of *H. pylori* against IBD in Mediterranean populations is less than in both East Asian and European counterparts (though only the former difference was statistically significant). Although this might suggest that the protective effect of diet is questionable, our meta-analysis did not explicitly incorporate dietary information and any putative effects of diet might be confounded in such a broad-scale analysis. Nevertheless, this does raise the need for further research into the role of diet on incidence of IBD. Although numerous studies have been conducted on recommended diets for prevention of IBD, there is still no consensus as to which is optimal [79]. Robust clinical trials have been limited by challenges such as defining the diet intervention, blinding, measuring intake and adherence over an extended period of time [79].

In conclusion, our meta-analysis supports previous findings of a protective effect of *H. pylori* infection on the risk of IBD, though we are the first to confirm that this protective effect is significantly stronger for CD compared to UC and for East Asian compared to Mediterranean populations. Our study is not without limitations and further primary research is warranted to evaluate genetic variability, pathogenesis, immunologic response, and environmental and dietary factors. A potential avenue for extending our study involves broadening the inclusion criteria to gain further insight into the regional variation of the protective effects of *H. pylori* on IBD.

Summary Box

What is already known:

- *Helicobacter pylori (H. pylori)* infection appears to have a protective effect against inflammatory bowel disease (IBD)
- *H. pylori* appears to be more protective against Crohn's disease (CD) than ulcerative colitis (UC), and in East Asian vs. western regions, though these have yet to be formally compared using hypothesis tests

What the new findings are:

- Our meta-analysis found a significant negative association between *H. pylori* infection and IBD, which varies by both IBD subtype (CD, UC) and geographic region (East Asia, Europe, Mediterranean)
- *H. pylori* infection provides significantly more protection against CD compared with UC
- *H. pylori* infection provides significantly more protection against IBD in East Asian compared to Mediterranean regions

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bowel diseases. A case-control study. *J Gastrointestin Liver Dis* 2018;**27**:119-125.

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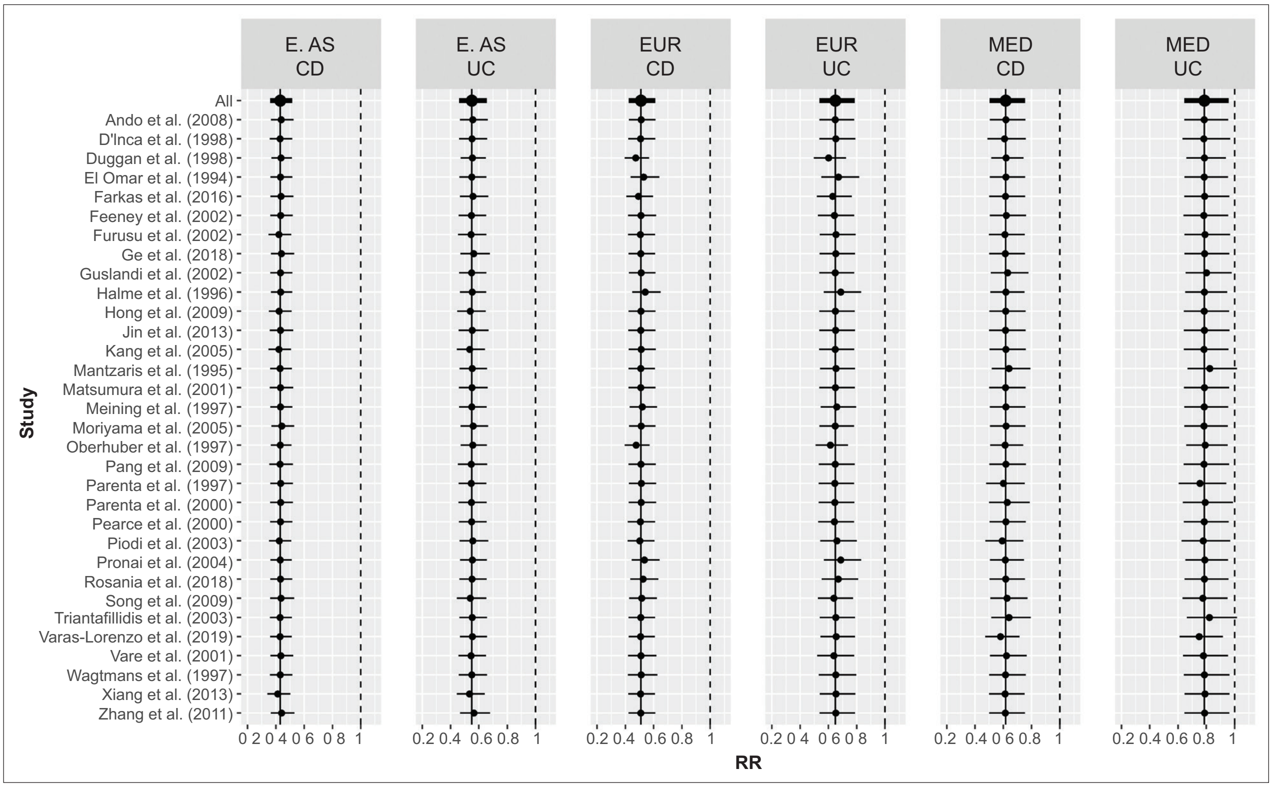
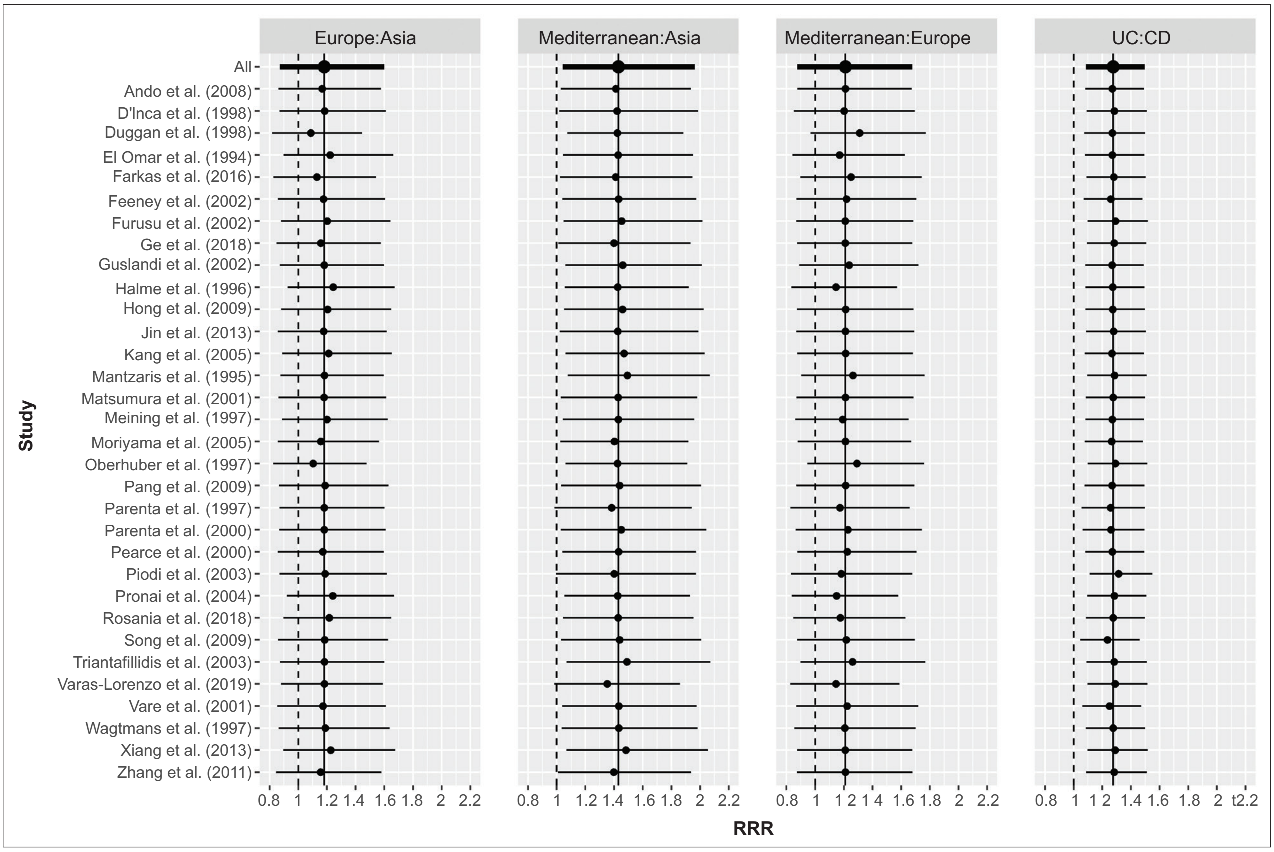
Supplementary material



Supplementary Figure 1 Leave-one-out sensitivity analysis for model predictions (summary effects expressed as relative risk [RR] with 95% confidence intervals) by region and subtype. The uppermost RR corresponds to summary effects presented in the paper, including all 32 studies in the meta-regression model. The remaining RR are model predictions with the corresponding study on the y-axis omitted. Vertical dashed lines are shown at RR=1 to indicate the null effect (confidence intervals that do not include 1 are statistically significant at P<0.05). The solid vertical lines represent the summary effect for all studies to help illustrate the displacement when each study is omitted. Under no circumstances does omitting a study result in a significant deviation from the overall summary effect. In 2 instances, the summary effect for Mediterranean-UC becomes (just) non-significant (Mantzaris *et al* 1995 and Triantafillidis *et al* 2003)



Supplementary Figure 2 Leave-one-out sensitivity analysis for model contrasts (expressed as ratios of relative risk [RRR] with 95% confidence intervals) for pre-specified contrasts of moderator variables (region and subtype) included in our meta-regression model. Confidence intervals are adjusted for simultaneous inference using the single-step procedure. RRR represent average effects, pooled over levels of the other moderator in the model. The uppermost RRR corresponds to those presented in the paper, including all 32 studies in the meta-regression model. The remaining RRR are contrasts with the corresponding study on the y-axis omitted. Vertical dashed lines are shown at RRR=1 to indicate the null effect (confidence intervals that do not include 1 are statistically significant at P<0.05). The solid vertical lines represent the RRR for all studies to help illustrate the displacement when each study is omitted. Under no circumstances does omitting a study result in a significant deviation from the overall summary effect (RRR). In 2 instances, the RRR for Mediterranean: Asia becomes (just) non-significant (Parenta *et al* 1997 and Varas-Lorenzo *et al* 2019)

Supplementary R code

```
# 1. Introduction ----
# R script to perform analyses reported in:
# Rabbiaatul Addawiyah Imawana, Daniel Robert Smith & Michaela Louise Goodson (2020)
# The relationship between Inflammatory Bowel Disease and Helicobacter pylori across
# East Asian, European and Mediterranean countries: a meta-analysis.
# Annals of Gastroenterology.

# R Code written by:
# Daniel R Smith; daniel.smith6@newcastle.edu.my
# Rabbiaatul A B Imawana; R.A.Binti-Imawana2@newcastle.ac.uk
# May-24-2020

# 2. Libraries ----
library(metafor) # Performing meta-analysis
library(plyr) # Manipulating data
library(ggplot2) # Visualising results
library(multcomp) # Performing contrasts
library(gridExtra) # Enhancing plots
library(dplyr) # Manipulating data
library(stringr) # Character string manipulation

# 3. Set seed ----
set.seed(1234) # To reproduce results

# 4. Functions ----
# Function for selected model (main effects only)
my_model <- function(...) {
  model <-
    rma.mv(
      ...,
      yi = yi,
      V = vi,
      mods =  ~ region + IBD.subtype,
      random =  ~ 1 | study / id,
      method = 'ML'
    )
  return(model)
}

# Function to compute relative risks and confidence intervals for each region x subtype combination
pred_fun_rr <- function(...) {
  p <- predict(..., transf = exp, digits = 2)
  df1 <- data.frame(subtype.region = rownames(my_dummy_matrix), p)
  df2 <- as.data.frame(str_split_fixed(df1$subtype.region, "_", 2))
  names(df2) <- c('subtype', 'region')
  df3 <- cbind(df2, df1)
  return(df3)
}
# Function to compute contrasts and confidence intervals (ratio of relative risks)
cont_fun_rr <- function(...) {
  cont <- summary(glht(...), test = adjusted("single-step"))
  conf <- confint(cont)
  pval <- conf$test$pvalues
  df1 <- data.frame(exp(conf$confint),pval=pval)
  df2 <- data.frame(contrast = rownames(df1), df1)
  return(df2)
}

# Function to perform leave-one-out sensitivity analysis for predictions and contrasts
sen_fun <- function(..., data, study_omit) {
  model <- my_model(..., data = data[!data$study == study_omit, ])
  preds1 <- pred_fun_rr(..., model, my_dummy_matrix)
  preds2 <- data.frame(study = study_omit, preds1)
  i <- sapply(preds2, is.factor)
  preds2[i] <- lapply(preds2[i], as.character)
  cont1 <- cont_fun_rr(..., model, my_contrast_matrix)
  cont2 <- data.frame(study = study_omit, cont1)
  j <- sapply(cont2, is.factor)
  cont2[j] <- lapply(cont2[j], as.character)
  mylistout <- list(preds = preds2, contrasts = cont2)
  return(mylistout)
}

# 5. Load & format data ----
# Read in data from master csv file
df <- read.csv(file = "IBD_Hpylori_master.csv")

# Tidy up study names
df$study <-
  as.factor(word(df$name, 1, sep = "_"))

# 6. Statistical analysis ----
# Compute effeCt sizes (relative risks)
es <-
  summary(
    escalc(
      measure = "RR",
      ai = Hppov.IBD,
      bi = Hpneg.IBD,
      ci = Hppov.control,
      di = Hpneg.control,
      data = df
    )
  )

# add observation id column
es$id <- 1:nrow(es)

# fit full mixed effets model including interaction term
mod1 <-
  rma.mv(
    yi = yi,
    V = vi,
    mods =  ~ region * IBD.subtype,
    random =  ~ 1 |
      study / id,
```

```
    data = es,
    method = 'ML'
  )   # includes interaction effect

# fit reduced form model excluding interaction term (i.e. only main effects)
mod2 <- update(mod1, mods =  ~ region + IBD.subtype)

# perfom a liklihood ratio test of full and reduced models and print results
writeLines("Liklihood ratio test of mod1 (full) and mod2 (reduced) models.....")
print(anova(mod1, mod2))
cat(' ', sep="\n\n")

# print results for mod2 (selected model for paper)
writeLines("Model used in paper.....")
print(mod2)
cat(' ', sep="\n\n")

# perform Wald test for region
writeLines("Wald test for region...")
print(anova(mod2,btt=2:3))
cat(' ', sep="\n\n")

# perform wald test for subtype
writeLines("Wald test for subtype...")
print(anova(mod2,btt=4))
cat(' ', sep="\n\n")

# Compute generalized I^2
# Formulae obtained from: http://www.metafor-project.org/doku.php/tips:i2_multilevel_multivariate
W <- diag(1 / es$vi)
X <- model.matrix(mod2)
P <- W - W %*% X %*% solve(t(X) %*% W %*% X) %*% t(X) %*% W
writeLines("Overall I^2=.....")
print(100 * sum(mod2$sigma2) / (sum(mod2$sigma2) + (mod2$k - mod2$p) / sum(diag(P))))
cat(' ', sep="\n\n")

# Define dummy matrices for predictions and contrasts
my_contrast_matrix <-
  rbind(
    'UC:CD' = c(0, 0, 0, 1),
    'Europe:Asia' = c(0, 1, 0, 0),
    'Mediterranean:Asia' = c(0, 0, 1, 0),
    'Mediterranean:Europe' = c(0, -1, 1, 0)
  )

my_dummy_matrix <-
  rbind(
    "CD_E. AS" = c(0, 0, 0),
    "CD_EUR" = c(1, 0, 0),
    "CD_MED" = c(0, 1, 0),
    "UC_E. AS" = c(0, 0, 1),
    "UC_EUR" = c(1, 0, 1),
    "UC_MED" = c(0, 1, 1)
  )

# Compute and print relative risks for region x subtype combinations
df_rr_all_mods_comb <- pred_fun_rr(mod2, my_dummy_matrix)
df_rr_all_mods_comb$study <-
  'All' # 'All' required as indicator for sensitivity analysis that follows...
writeLines('Relative risk and confidence intervals by region and subtype.....')
print(df_rr_all_mods_comb)
cat(' ', sep="\n\n")

# Compute and print contrasts (ratios of relative risks)
df_rrr_cont <- cont_fun_rr(mod2, my_contrast_matrix)
df_rrr_cont$study <-
  'All'  # mark that used all studies so can include on leave one out plot below...
writeLines('Ratios of relative risks and confidence intervals')
print(df_rrr_cont)
cat(' ', sep="\n\n")

# 7. Diagnostics ----
# Sensitivity analysis (leave-one-out)

# Define list of studies
ls_studies <- as.list(levels(es$study))

# Fit model to orginal data, iteratively omitting one study per loop. Store predictions and contrasts in a list
sen_list <-
  lapply(ls_studies, function(x)
    sen_fun(data = es, study_omit = x))

# Subset list for predictions and bind to make dataframe
sen_df_preds <- bind_rows(lapply(sen_list, '[[', "preds"))

# Subset list for contrasts and bind to make dataframe
sen_df_contrasts <- bind_rows(lapply(sen_list, '[[', "contrasts"))

# Create dataframe for predictions plot by binding original and leave one out analyses
sen_df_preds_plot <- rbind(sen_df_preds, df_rr_all_mods_comb)
sen_df_preds_plot$study <- as.factor(sen_df_preds_plot$study)

# Create dataframe for contrasts plot by binding original and leave one out analyses
sen_df_contrasts_plot <- rbind(sen_df_contrasts, df_rrr_cont)
sen_df_contrasts_plot$study <- as.factor(sen_df_contrasts_plot$study)
# Pseudo egger test for small-study bias
# Note the addition of study variances (vi) to the model
mod2_egg <- update(mod2, mods = ~ vi + region + IBD.subtype)
writeLines("Pseudo Egger-test for reduced model (see P value corresponding to intercept).....")
print(mod2_egg)
cat(' ', sep="\n\n")
```

```
# 8. Plots ----
# Prepare dataframe for plotting
df_sub1 <-
  subset(df_rr_all_mods_comb,
       select = c(study, subtype, region, pred, ci.lb, ci.ub))
names(df_sub1)[4] <- 'estimate'
df_sub2 <-
  subset(es, select = c(study, IBD.subtype, region, yi, ci.lb, ci.ub))
names(df_sub2)[c(2, 4)] <- c('subtype', 'estimate')
df_sub2_exp <- data.frame(df_sub2[1:3], lapply(df_sub2[4:6],
    exp))
df_sub3 <- rbind(df_sub1, df_sub2_exp)


df_sub3$region <-
  mapvalues(
    df_sub3$region,
     from = c("E. AS", "EUR", "MED", "East Asia","Europe","Me
     diterranean"),
    to = c(
      "East Asia" ,
      "Europe",
      "Mediterranean",
      "East Asia",
      "Europe",
      "Mediterranean"
    ),
    warn_missing = TRUE
  )

# Forest plot of relative risks by region and subtype
tiff(
  file = "Figure_2.tiff",
  width = 6500,
  height = 3500,
  res = 600
)
print(
  ggplot(df_sub3[!df_sub3$study == 'All', ], aes(x = study, y =
     estimate)) + geom_point() + facet_grid(~ region +
                                                           subtype) +
    geom_errorbar(aes(
      ymin = ci.lb, ymax = ci.ub, width = 0
    )) +
    geom_point(
      data = df_sub3[df_sub3$study == 'All', ],
      aes(x = study, y = estimate),
      colour = "black",
      size = 1.5) +
    geom_errorbar(
      data = df_sub3[df_sub3$study == 'All', ],
      aes(ymin = ci.lb, ymax = ci.ub, width = 0),
      colour = "black",
      size = 1
    ) +
    coord_flip() +
    geom_hline(yintercept = 1, lty = 2) +
      scale_x_discrete(name = "Study", limits = rev(levels(df_
     sub3$study))) +
          scale_y_continuous(name = 'RR') + # ,
    breaks=c(seq(0.2,1.2,0.2)), labels=c(seq(0.2,1.2,0.2)),limits
    =c(0.2,1.1)) +
    theme(
      axis.text = element_text(size = 8),
      axis.title = element_text(size = 14, face = "bold"),
      strip.text.x = element_text(size = 13),
      panel.spacing = unit(1.5, "lines")
    )
)
dev.off()

# Contrasts (ratio's of relative risks)
tiff(
  file = "Figure_3.tiff",
  width = 4000,
  height = 4000,
  res = 600
)
print(
  ggplot(data = df_rrr_cont, aes(
    x = rownames(df_rrr_cont),
    y = Estimate,
    ymin = lwr,
    ymax = upr
  )) +
    geom_pointrange(size = 1) +
    geom_hline(yintercept = 1, lty = 2) +
    coord_flip() +
    ylim(0.8, 2) +
    xlab("Contrast") +
    ylab("RRR (95% CI)") +
    theme(
      axis.text = element_text(size = 12),
      axis.title = element_text(size = 14, face = "bold")
    )
)
dev.off()

# Sensitivity analysis relative risks
tiff(
  file = "Figure_1_supplementary_information.tiff",
  width = 6500,
  height = 4000,
  res = 600
)
print(
      ggplot(sen_df_preds_plot[!sen_df_preds_plot$study ==
    'All', ], aes(x = study, y =
                                                pred)) + geom_point()
    + facet_grid(~ region + subtype) +
    geom_errorbar(aes(
      ymin = ci.lb, ymax = ci.ub, width = 0
    )) +
    geom_point(
        data = sen_df_preds_plot[sen_df_preds_plot$study ==
    'All', ],
      aes(x = study, y = pred),
      colour = "black",
```

```
    size = 3
  ) +
  geom_errorbar(
      data = sen_df_preds_plot[sen_df_preds_plot$study ==
    'All', ],
    aes(ymin = ci.lb, ymax = ci.ub, width = 0),
    colour = "black",
    size = 1.5
  ) +
  coord_flip() +
  geom_hline(yintercept = 1, lty = 2) +
          geom_hline(data=sen_df_preds_plot[sen_df_preds_
   plot$study == 'All',],aes(yintercept=pred),lty = 1) +
  scale_x_discrete(name = "Study", limits = rev(levels(
    sen_df_preds_plot$study
  ))) +
  scale_y_continuous(
    name = 'RR',
    breaks = c(seq(0.2, 1.2, 0.2)),
    labels = c(seq(0.2, 1.2, 0.2)),
    limits = c(0.2, 1.1)
  ) +
  theme(
    axis.text = element_text(size = 10),
    axis.title = element_text(size = 14, face = "bold"),
    strip.text.x = element_text(size = 14),
    panel.spacing = unit(1.5, "lines")
  )
)
dev.off()

# Sensitivity analysis contrasts
tiff(
  file = "Figure_2_supplementary_information.tiff",
  width = 6000,
  height = 4000,
  res = 600
)
print(
   ggplot(sen_df_contrasts_plot[!sen_df_contrasts_plot$study
    == 'All', ], aes(x =
                                           study, y = Estimate))
    + geom_point() + facet_grid(. ~ contrast) +
  geom_errorbar(aes(
    ymin = lwr, ymax = upr, width = 0
  )) +
  geom_point(
    data = sen_df_contrasts_plot[sen_df_contrasts_plot$study
    == 'All', ],
    aes(x = study, y = Estimate),
    colour = "black",
    size = 3
  ) +
  geom_errorbar(
    data = sen_df_contrasts_plot[sen_df_contrasts_plot$study
    == 'All', ],
    aes(ymin = lwr, ymax = upr, width = 0),
    colour = "black",
    size = 1.5
  ) +
  coord_flip() +
  geom_hline(yintercept = 1, lty = 2) +
   geom_hline(data=sen_df_contrasts_plot[sen_df_contrasts_
    plot$study == 'All', ],aes(yintercept=Estimate),lty = 1) +
  scale_x_discrete(name = "Study", limits = rev(levels(
    sen_df_contrasts_plot$study
  ))) +
  scale_y_continuous(
    name = 'RRR',
    breaks = c(seq(0.8, 2.2, 0.2)),
    labels = c(seq(0.8, 2.2, 0.2)),
    limits = c(0.8, 2.2)
  ) +
  theme(
    axis.text = element_text(size = 10),
    axis.title = element_text(size = 14, face = "bold"),
    strip.text.x = element_text(size = 12),
    panel.spacing = unit(1.5, "lines")
  )
)
dev.off()

# Standard funnel plot
tiff(
  file = "Figure_4.tiff",
  width = 4000,
  height = 4000,
  res = 600
)
funnel(mod2)
dev.off()

# Contour enhanced funnel plot (centered at 0)
tiff(
  file = "Figure_5.tiff",
  width = 4000,
  height = 4000,
  res = 600
)
funnel(
  mod2_egg,
  level = c(90, 95, 99),
  shade = c("white", "gray40", "gray50"),
  refline = 0,
  legend = FALSE
)
p<-recordPlot()
legend("topright",
legend=c("0.10 < P < 1.00",
      "0.05 < P \u2264 0.10",
      "0.01 < P \u2264 0.05",
      "0.00 < P \u2264 0.01"),
      fill = c("white", "gray40", "gray50", "gray"),
  pt.cex=1.2, cex=1.2)
dev.off()
```