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Complete List of Authors:	Hao, Ning-Bo He, Ya-Fei Luo, Gang Yong, Xin Zhang, Yao yang, shiming; Department of Gastroenterology, Xinqiao Hospital, Third Military Medical University
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Macrophage migration inhibitory factor -173 G/C polymorphism and the risk of inflammatory bowel disease: A meta-analysis

Ning-Bo Hao[1], Ya Fei He[1], Gang Luo[1], Xin Yong[1], Yao Zhang[2, 3,*] and Shi-Ming Yang[1, 4, *]

[1] Department of Gastroenterology, Xinqiao Hospital, Third Military Medical University; Chongqing 400038, P.R. China

[2] Department of Epidemiology, Third Military Medical University, Chongqing 400038, P.R. China

[3] The Evidence Based Medicine and Clinic Epidemiology Center, Third Military Medical University, Chongqing, 400038, P.R. China

[4] Chongqing Key Laboratory for Diseases Proteomics, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

[*] To whom requests for reprints should be addressed at the Department of Gastroenterology, Third Military Medical University, Chongqing 400037, China. Phone: 86-023-68754678; E-mail: shimingyang@yahoo.com or the Department of Epidemiology, Third Military Medical University, Chongqing 400038, P. R. China sydzy2003@yahoo.com.cn

Running Title: MIF -173 G/C polymorphism and the risk of IBD

Abbreviations: MIF, macrophage migration inhibitory factor; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; OR, odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

Abstract

Objective: To determine whether macrophage migration inhibitory factor (MIF) gene polymorphism is associated with the risk of inflammatory bowel disease (IBD).

Design: System review and meta-analysis.

Methods: MEDLINE, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical Literature database (CBM) were searched for the case-control trails for MIF and IBD. All the studies included into this manuscript met the inclusion and exclusion criteria. An odds ratio (OR) analysis using a 95% confidence interval (CI) was employed to assess the association of the MIF-173 G/C polymorphism with IBD susceptibility.

Results: There was a significant association between the MIF-173 G/C gene polymorphism and IBD in the total population under both the recessive model (CC vs. GC+GG) (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and the codominant model (CC vs. GG) (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). In the stratified analysis by ethnicity, significantly increased risks were observed for Asians using the recessive (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and codominant models (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). Within the subgroups of UC and CD, significant differences were observed regarding UC using the recessive (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and codominant models (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). In the stratified analysis by ethnicity for UC, significant differences were observed regarding CC in Asians vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity).

Conclusion: The meta-analysis suggested that the MIF-173 G/C polymorphism contributed to the susceptibility of IBD. When considering the subgroups of ethnicity and UC and CD, the results suggested that the polymorphism is more significant for UC in Asians.

Key Words: Crohn's disease, gene polymorphism, inflammatory bowel disease, macrophage migration inhibitory factor, ulcerative colitis.

Introduction

Inflammatory bowel disease (IBD) currently represents one of the most common health problems worldwide. The highest incidence rates and prevalence of ulcerative colitis (UC) and Crohn's disease (CD) have been reported in northern Europe, the UK, and North America [1]. In addition, in low-incidence areas, such as Asia, southern Europe, and most developing countries, rates also continue to rise [2]. However, the etiology and pathogenesis are still unknown.

Our current understanding of IBD is a complex disease with a number of contributing factors, such as genetic predisposition, environmental factors, intestinal microbial flora, and an aberrant immune response [1]. It has been demonstrated that many IBD patients have a dysregulated intestinal mucosal T-cell-mediated immune response, specifically involving CD4 T helper type-1 (Th1) lymphocytes, which leads to the production of Th1-associated pro-inflammatory cytokines, such as interferon-γ (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-alpha (TNF-α)[3].

In addition, another cytokine, macrophage migration inhibitory factor (MIF), is considered to play a critical role in immunity and inflammation. MIF is secreted by a series of immune cells, such as macrophages, dendritic cells, lymphocytes, neutrophils and pituitary cells[4 5]. Once secreted, MIF regulates a broad range of immune and inflammatory activities, including the induction of inflammatory cytokines, such as TNF-α, IFN-γ, IL-1β, IL-12, IL-6 and, CXCL8 (also known as IL-8), among others [6]. However, if the MIF gene is mutated, it will disturb the immune balance in the microenvironment. It has been demonstrated that MIF gene

mutation is associated with many autoimmune diseases, such as rheumatoid arthritis, glomerulonephritis, and inflammatory bowel diseases [7-11]. MIF genotyping studies have focused on the -794 $CATT_{(5–8)}$ microsatellite and the -173 G/C polymorphism[12]. Donn et al first reported that the MIF 173 polymorphism is a risk factor for juvenile idiopathic arthritis [13]. Consequently, Baugh et al reported the association between the -794 $CATT_{(5–8)}$ microsatellite and disease severity in patients with rheumatoid arthritis[14]. In addition, it has also been reported that the MIF gene polymorphism is a risk factor for other immune diseases, such as atrophy, asthma and sarcoidosis in erythema nodosum patients[12]. Therefore, it is important to explore whether MIF gene polymorphisms are associated with IBD. However, the research results are not in agreement. Thomas and colleagues found that there was no significant difference between IBD patients and controls with the MIF gene type [15]. In other articles, the MIF gene polymorphism has been reported to be a risk factor for IBD [16 17]. Because most of the articles on MIF gene polymorphisms and IBD studied the -173 G/C polymorphism, we performed a meta-analysis to determine whether the MIF -173 G/C polymorphism is a risk factor for IBD. Our analysis reveals that the MIF -173 G/C polymorphism is a risk factor for IBD, especially for UC in Asians.

Materials and Methods

Search Strategy

This meta-analysis followed the proposal of the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group and was performed by searching PubMed, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical

Literature database (CBM) (last search updated in April, 2013) [18]. The search strategy included the following terms: (macrophage migration inhibitory factor [MeSH] or MIF [TEXT WORD] and inflammatory bowel disease [MeSH] or ulcerative colitis [TEXT WORD] or Crohn's disease [TEXT WORD] or IBD [TEXT WORD] or UC [TEXT WORD] or CD [TEXT WORD]). Searches also included scanning reference lists in relevant articles and conference proceedings as well as correspondence with authors when additional data were required. Two reviewers (Hao and He) independently screened the titles and abstracts of each identified reference and categorized papers based on the full text to evaluate their eligibility for inclusion.

Inclusion and Exclusion Criteria

The inclusion criteria for primary studies were as follows: (1) the article evaluated MIF gene polymorphisms and IBD risk; (2) the article included case–control studies or a nested case–control study; (3) the article supplied the number of individual genotypes for the MIF polymorphisms in inflammatory bowel disease cases and controls; and (4) the article demonstrated that the distribution of genotypes among controls was in Hardy–Weinberg equilibrium. In addition, the following exclusion criteria were used. the studies were excluded when 1) it did not provide detailed data such as presented in abstracts, meeting reports and reviews; 2) the studies were repeated or overlapped other publications; 3) the genotype frequency was not reported; and 4) the controls do not meet the assumptions for HWE.

Data Extraction

No paper was included if it did not meet the four inclusion criteria. When the

same study results appeared in several papers, only one study was used in this meta-analysis. Table 1 lists the characteristics of the extracted data, including the first author's name, publication date, region of study, ethnicity of the sample population, number of genotypes, and the total number of cases and controls. The study regions included China, Japan, Spain, Germany and Poland. Data extraction was independently performed by two individuals (Hao and He), and any disagreement was resolved by consensus or by consultation with additional reviewers (Zhang and Yang).

Qualitative Assessment

Quality assessment was performed with the Newcastle-Ottawa quality assessment scale (NOS) for case-control studies. A 'star system' was used to judge data quality based on three broad perspectives: selection, comparability and outcome of interest for case-control studies. Star counts were totaled to compare the study quality in a quantitative fashion[19]. Based on these criteria, the content validity was evaluated by Hao and He, and any disagreement was resolved via discussions between Hao and Luo or with the other authors (Zhang and Yang) for adjudication.

Statistical Analysis

All statistical tests were performed using Revman 5.0 software. Deviations from Hardy–Weinberg equilibrium (HWE) were calculated for the control groups by χ^2 goodness-of-fit. The association between the MIF -173G/C gene polymorphism and IBD was compared by the odds ratio (OR) and the corresponding 95% confidence interval (CI) between the case and control groups. The statistical significance of the summary OR was determined with the Z test, and P less than 0.05 was considered as

statistically significant. The genetic models evaluated for the polymorphism were the dominant model (GC+CC vs. GG), the recessive model (CC vs. GC+GG), the allelic gene model (C vs. G) and the codominant model (CC vs. GG and GC vs. GG).

The heterogeneity between studies was determined by the Chi-square-based Q-test. A P value greater than 0.05 for the Q-test indicated a lack of heterogeneity among the studies. If there was a lack of heterogeneity, the pooled OR estimate of each study was calculated using a fixed-effects model (the Mantel–Haenszel method); otherwise, a random-effects model (the DerSimonian and Laird method) was used [20 21]. In addition, subgroup analysis stratified by ethnicity and different disease types was also performed. The potential publication bias was estimated by a funnel plot. Egger's linear regression test on the natural logarithm scale of the OR was used to assess the funnel plot asymmetry; the significance was set at the $P<0.05$ level [22].

Results

Study Characteristics

Figure 1 outlines our search process. First, a total of 123 articles were collected after the initial search with the key words listed previously. After reading the title and abstract, 12 reviews and 1 editorial were excluded. Next, 99 articles were excluded for no association with IBD and the MIF -173 G/C polymorphism. The remaining 11 articles were identified for full-text review, and 4 articles were excluded because of repeated publications. In addition, 1 article was excluded because it deviated from HWE [23]. Finally, 6 articles met our inclusion criteria and were used for the meta-analysis with 2084 cases and 2288 controls [15-17 24-26]. The basic characteristics of

these articles are listed in Table 1. Of the eligible studies, 3/6 articles researched the association of the MIF-173 gene polymorphism with both UC and CD. The remaining 3/6 articles only researched the association of the MIF -173 G/C polymorphism with UC. In total, 3/6 studies were performed in Europeans, and 3/6 were performed in Asians. All studies were cross-sectional case-control studies with sufficient data to calculate the possible relationship between the MIF -173 G/C polymorphism and IBD. All studies were published in English. The distribution of the MIF -173 gene type in IBD, UC and CD is listed in Table 2.

Quantitative Data Synthesis

Table 3 lists the primary results. In the total population, we found a significant difference between the MIF -173 G/C gene polymorphism and the risk of IBD for two variants: CC vs. GC+GG (OR=1.5, CI =1.07-2.14, P=0.02 for heterogeneity) and CC vs. GG (OR=1.54, CI=1.09-2.24, P=0.01 for heterogeneity) (Figures 2 A， B， C and D). No significant difference was observed for the variants of GC+CC vs. GG, GC vs. GG or the allele C vs. G (Table 3).

In the stratified analysis by ethnicity, a significantly increased risk was observed in Asians for CC vs. GC+GG (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and CC vs. GG (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). (Figure 2 A, B) However, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.36, CI=0.86-2.15, P=0.19 for heterogeneity) or CC vs. GG (OR=1.42, CI=0.89-2.24, P=0.14 for heterogeneity).

Because IBD includes UC and CD, we determined whether the role of the MIF

-173 G/C gene polymorphism was different between the two diseases. Therefore, we analyzed subgroups of UC and CD. Significant differences were observed in UC for CC vs. GC+GG (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and CC vs. GG (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). (Figure 2 C and D) However, no significant differences were found in CD for CC vs. GC+GG (OR=1.41, CI=0.85-2.36, P=0.19 for heterogeneity) or CC vs. GG (OR=1.44, CI=0.86-2.40, P=0.16 for heterogeneity).

Although IBD includes UC and CD, the cases of IBD in Asians included in our data were mainly UC, and only 15 cases were CD. This may influence the accuracy of the meta-analysis results in the subgroup of ethnicity. Therefore, we analyzed the association of the MIF -173 G/C polymorphism with UC in the subgroup of ethnicity. As shown in Figure 3A, a significant difference was observed in Asians for CC vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity). Similar to previous results, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.47, CI=0.85-2.55, P=0.17 for heterogeneity).

Sensitivity Analyses and Publication Bias

Sensitivity analyses were performed to assess the influence of each individual study on the pooled ORs by the systematic omission of the individual studies from the analyses. the corresponding pooled OR was not materially altered. Begg's funnel plot and the Egger's test were performed to assess the publication bias of the literature. As shown in Figure 3B, the shape of the funnel plot did not reveal obvious asymmetry. The Egger's test was used to provide statistical evidence of funnel plot symmetry. The

results still did not suggest any evidence of publication bias (data not shown).

Discussion

The human MIF gene, which is located on chromosome 22q11.2, is short; it is composed of 3 exons of 205, 173, and 183 bp and 2 introns of 189 and 95 bp [12 27 28]. Four polymorphisms of the human MIF gene have been reported, including a 5–8 CATT tetranucleotide repeat at position -794 $CATT_{(5–8)}$ and 3 single-nucleotide polymorphisms (SNPs) at positions -173 G/C, +254 T/C, and +656 C/G [13 14 29]. However, in IBD, studies on MIF gene polymorphisms have mainly focused on the -173 G/C SNP. Therefore, in this meta-analysis, we mainly discussed the association of the MIF -173 G/C gene polymorphism with the susceptibility to IBD.

In the current meta-analysis of 6 studies consisting of 2084 cases and 2288 controls, we found that the MIF -173 G/C gene polymorphism is significantly associated with IBD susceptibility in both the recessive model (OR: 1.5) and codominant model (OR: 1.54), while no significant associations were found in the dominant model or the allelic model. The results indicated that the MIF -173 G/C polymorphism was a conspicuous high-risk factor for developing IBD in the overall study population.

The second finding of this meta-analysis is that in the subgroup of ethnicity, the MIF -173 G/C gene polymorphism was significantly different in Asians in the recessive (OR: 1.75) and codominant (OR: 1.74) models, while no significant differences were found in Europeans. This finding is consistent with previous results that a gene polymorphism does not have the same effect in different ethnicities. For

example, the TNF-α308A gene polymorphism plays an important role in Asian populations, while no conclusive data on this association exist in European patients [30]. In addition, the best studied genetic variant, a nucleotide oligomerization domain (NOD)2 polymorphism, is present in up to 20% of patients with CD in White and Jewish populations, but major disease-associated variants have not been detected in individuals of Asian descent with CD [31 32].

The third finding of this meta-analysis is that in the subgroup of the two diseases, UC and CD, significant differences were found in the recessive (OR: 1.60) and codominant (OR: 1.64) model for UC, while no difference was found in the recessive or codominant model for CD. This result suggests that the MIF -173 G/C polymorphism seems to be a risk factor for UC but not for CD. However, in another study, Zhang and colleagues confirmed that the peroxisome proliferator-activated receptor gamma (PPARγ) AlaAla genotype is a protective factor against the development of CD, especially in the European Caucasian population, while no significant association was found in the development of UC [33]. Both of these results indicated that the gene polymorphism has different effects in different diseases.

To further determine whether the MIF -173 gene polymorphism for UC was different in different ethnicities, we performed a meta-analysis of UC in the subgroup of ethnicity. A significant difference was found in the recessive model (OR: 1.73) in Asians, while no significant difference was found in Europeans. Although only three studies were included in the sub-group analysis, they all have high quality. So the results in the sub-group analysis were credible. This result suggests that the MIF -173

G/C polymorphism is a risk factor for UC in Asians but not in Europeans.

The present meta-analysis had several limitations that must be taken into consideration. First, the number of available studies that could be included in this meta-analysis was moderate. Therefore, the results could be influenced by factors such as random error. Second, the number of cases of CD in Asia included in this meta-analysis is low, which may influence the accuracy of the final results. Therefore, next, our laboratory will focus on research on the association between gene polymorphisms and CD in Asia. Third, the overall outcomes were based on individual unadjusted ORs, while a more precise evaluation should be adjusted by other potentially suspected factors, including age, sex, and environmental factors.

In summary, the current meta-analysis suggested that the MIF -173 G/C polymorphism contributed to the susceptibility of IBD. In the subgroups of ethnicity and UC and CD, the result suggested that this polymorphism was more significant for UC in Asians.

Acknowledgements

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Conflict of interest

The authors declare that they have no conflict of interest.

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Legends to Figures

Figure 1 Flow of study identification, inclusion, and exclusion.

Figure 2 Forest plot of inflammatory bowel disease risk associated with MIF 173 for (A) CC vs. GG+GC and (B) CC vs. GG for ethnicity and (C) CC vs. GC+GG and (D) CC vs. GG for UC and CD.

Figure 3 (A) Forest plot of ulcerative colitis risk associated with MIF 173 for CC vs. GG+GC for ethnicity; (B) Begg's funnel plot of the MIF 173 gene polymorphism and

inflammatory bowel disease risk for combined CC vs. GC+GG

Article Summary

Article Focus:

1. Examines whether MIF gene polymorphism is associated with IBD, UC or CD.
2. Do Asians and Europeans have different gene mutation points?
3. Are UC and CD caused by the same gene mutation points?

Key messages:

1. It was demonstrated that MIF gene polymorphism is associated with IBD.
2. In Asia, it was found that MIF gene polymorphism is associated with IBD, especially for UC. However, in European, no association was found.
3. In Asia, the main IBD patients were UC, while in European the main patients were CD.

Strengths and limitations of this study:

1. A large number of patients were concluded in this manuscript, which is important when examining the association between MIF and IBD.
2. All the articles included in this manuscript must conform that all controls was in Hardy–Weinberg equilibrium.
3. It still needs to demonstrate that MIF gene polymorphism was associated with IBD in other places such as Africa and South and North America.

Author contributorship statement

1. Hao,and He contributed to conception and design, acquisition of data, or analysis and interpretation of data.
2. Hao and Yong acquisition of data
3. Hao, He, Luo and Zhang analyses and interpreted the data.
4. Hao and Yang drafted the article.
5. All the authors listed in this manuscript approval to publish in ***BMJ OPEN***.

Table1 Characteristics of studies included in meta-analysis

First author	Years	Country	Ethnicity	Case number(n)	Control Number (n)	Newcastle-Ottawa Score	Genotyping
Griaga	2007	Germany	European	259	489	7/9	PCR-RFLP
Shiroeda	2010	Japan	Asian	111	209	6/9	PCR-SSCP
Fei	2008	China	Asian	99	142	9/9	PCR-RFLP tetra-primer ARMS
Oliver	2007	Spain	European	1295	887	8/9	PCR-RFLP
Przybyłowska	2011	Poland	European	99	123	6/9	PCR-RFLP
Nohara	2004	Japan	Asian	221	438	7/9	PCR-RFLP PCR-SSCP

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism， ARMS： amplification refractory mutation system

PCR-SSCP, polymerase chain reaction-single-strand conformation polymorphism，

Table 2: Genotypes and allele frequencies of MIF-173G/C genes in patients and controls

Author	Year	Case					Control					Sample size	HWE（P）
		GG	GC	CC	G	C	GG	GC	CC	G	C		
IBD													
Fei	2008	52	32	15	136	62	79	55	8	213	71	99/142	0.70
Griaga	2007	188	67	4	443	75	318	156	15	792	186	259/489	0.43
Nohara	2004	135	76	10	346	96	288	134	16	710	166	221/438	0.93
Shiroeda	2010	69	37	5	175	47	126	76	7	328	90	111/209	0.27
Przybyłowska	2011	66	31	2	163	35	99	23	1	221	25	99/123	0.79
Oliver	2007	907	343	45	2157	433	681	188	18	1550	224	1295/887	0.24
UC													
Fei	2008	44	27	13	115	53	79	55	8	213	71	84/142	0.70
Griaga	2007	72	28	2	172	32	318	156	15	792	186	102/489	0.43
Nohara	2004	135	76	10	346	96	288	134	16	710	166	221/438	0.93
Oliver	2007	441	171	22	1053	215	681	188	18	1550	224	634/887	0.24

Przybyłowska	2011	38	19	1	95	21	99	23	1	221	25	58/123	0.79
Shiroeda	2010	69	37	5	175	47	126	76	7	328	90	111/209	0.27
CD													
Fei	2008	8	5	2	21	9	79	55	8	213	71	15/142	0.70
Griaga	2007	116	39	2	271	43	318	156	15	792	186	157/489	0.43
Oliver	2007	466	172	23	1104	218	681	188	18	1550	224	661/887	0.24
Przybyłowska	2011	28	12	1	68	14	99	23	1	221	25	41/123	0.79

HWE: Hardy-Weinberg Equilibrium

Table 3 Summary of different comparative results

	Study	Sample size		No. of	Test of association			Model	Heterogeneity		
		case	control	studies	OR (95% CI)	*Z*	*P* Value		χ^2	*P* Value	I^2 (%)
GC+CC vs. GG	Overall	2084	2288	6	1.15（0.87-1.52）	0.97	0.33	R	17.04	0.004	71.0
	Asian	431	789	3	1.12 (0.88 – 1.44)	0.90	0.37	F	0.90	0.64	0.0
	European	1653	1499	3	1.23 (0.70 – 2.14)	0.71	0.48	R	15.75	0.0004	87.0
	UC	1210	2288	6	1.24 (1.06 – 1.44)	2.76	0.006	F	9.69	0.08	48.0
	CD	874	1641	4	1.13 (0.69 – 1.87)	0.49	0.62	F	11.46	0.009	74.0
CC vs. GC+GG	Overall	2084	2282	6	1.51(1.07-2.14)	2.34	0.02	F	6.72	0.24	26.0
	Asian	431	789	3	1.75(1.04 – 2.95)	2.10	0.04	F	2.21	0.33	9.0
	European	1653	1499	3	1.36 (0.86 – 2.15)	1.31	0.19	F	4.16	0.12	52.0
	UC	1210	2288	6	1.60 (1.09 – 2.35)	2.42	0.02	F	3.92	0.56	0.0
	CD	874	1641	4	1.41 (0.85 – 2.36)	1.32	0.19	F	3.91	0.27	23.0
CC vs. GG	Overall	1498	1656	6	1.54（1.09-2.24）	2.43	0.01	F	7.30	0.20	32.0
	Asian	286	524	3	1.74（1.02– 2.97）	2.04	0.04	F	1.72	0.42	0.0
	European	1212	1132	3	1.42 (0.89 – 2.24)	1.49	0.14	F	5.38	0.07	63.0
	UC	852	1656	6	1.64 (1.12 – 2.41)	2.52	0.01	F	3.88	0.57	0.0
	CD	646	1219	4	1.44 (0.86 – 2.40)	1.40	0.16	F	4.68	0.20	36.0
GC vs. GG	Overall	2003	1972	6	1.40（1.00-1.95)	1.97	0.05	R	18.46	0.002	73.0
	Asian	401	810	3	1.02 (0.79 – 1.31)	0.14	0.89	F	1.01	0.60	0.0
	European	1602	1465	3	1.21 (0.72 – 2.03)	0.73	0.47	R	12.77	0.002	84.0
	UC	1157	2223	6	1.20 (1.02 – 1.40)	2.20	0.03	F	9.64	0.09	48.0
	CD	846	1599	4	1.09 (0.69 – 1.74)	0.39	0.70	R	9.13	0.03	67.0
C vs. G	Overall	4168	4576	6	1.17 (0.91 – 1.50)	1.25	0.21	R	18.47	0.002	73.0
	Asian	862	1578	3	1.17 (0.96 – 1.43)	1.54	0.12	F	1.36	0.51	0.0
	European	3306	2998	3	1.20 (0.72 – 2.01)	0.70	0.49	R	17.04	0.0002	88.0
	UC	2420	4576	6	1.24 (1.09 – 1.41)	3.20	0.001	F	9.51	0.08	50.0
	CD	1748	3282	4	1.16(0.73 – 1.83)	0.63	0.53	R	12.78	0.005	77.0

* vs.: versus; R: random-effects model; F: fixed-effects model.

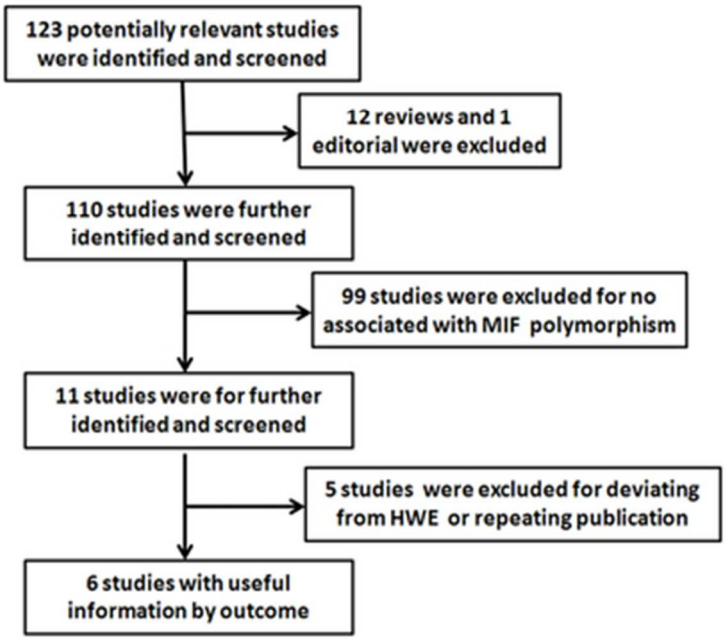

Figure 1

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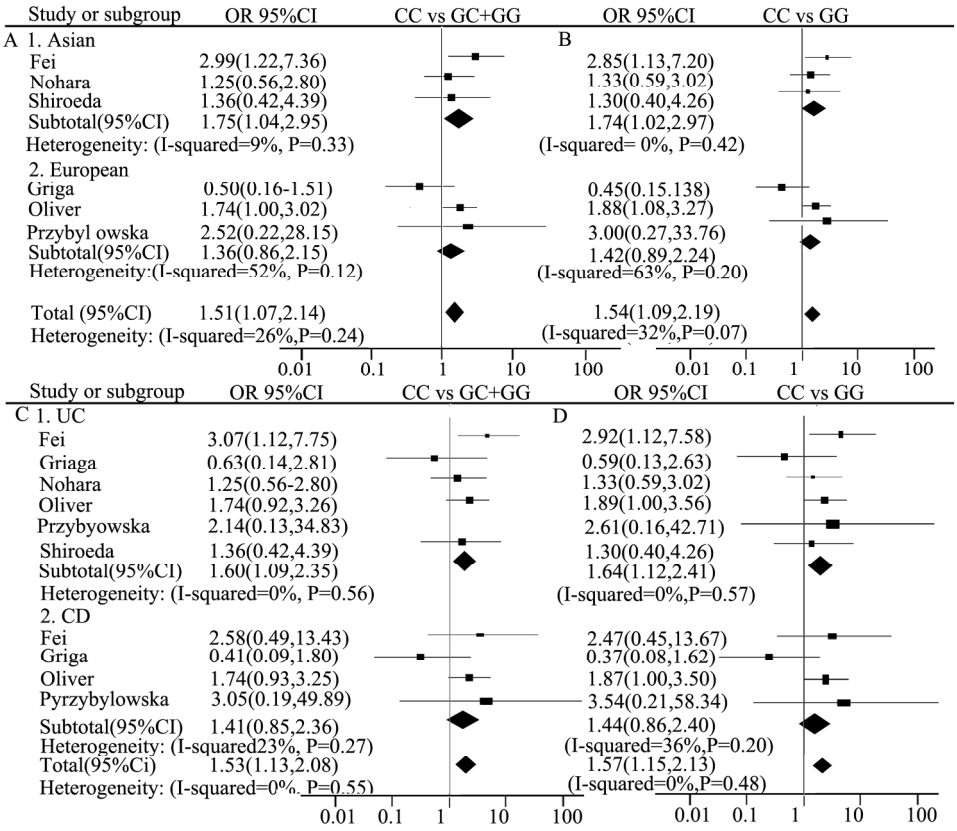

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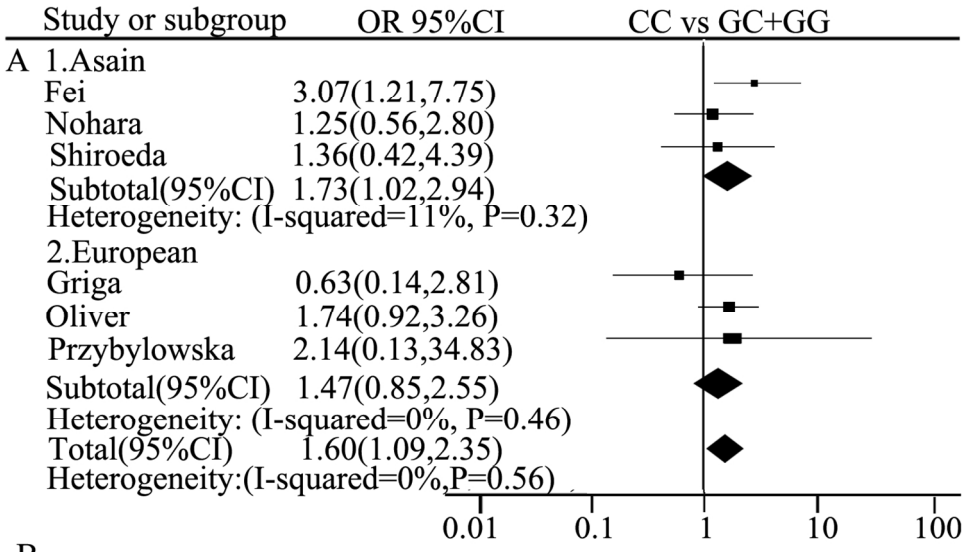

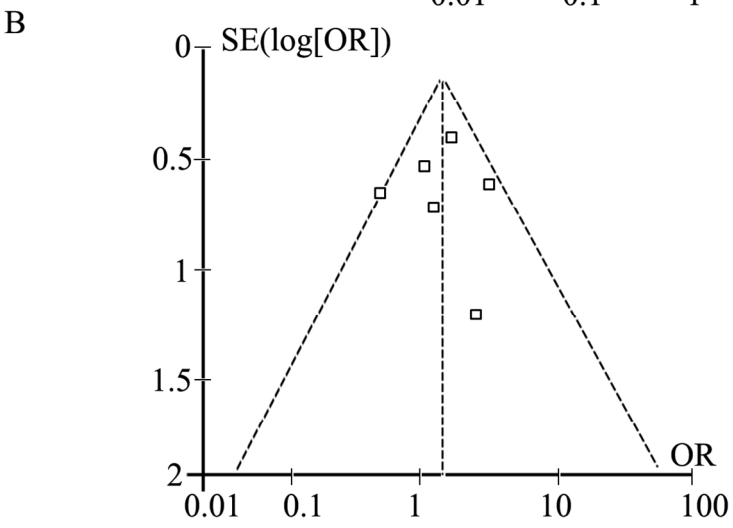

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Macrophage migration inhibitory factor polymorphism and the risk of Ulcerative Colitis and Crohn's Disease in Asian and European: A meta-analysis

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Macrophage migration inhibitory factor polymorphism and the risk of Ulcerative Colitis and Crohn's Disease in Asian and European: A meta-analysis

Ning-Bo Hao[1], Ya Fei He[1], Gang Luo[1], Xin Yong[1], Yao Zhang[2, 3,*] and Shi-Ming Yang[1, 4, *]

[1] Department of Gastroenterology, Xinqiao Hospital, Third Military Medical University; Chongqing 400038, P.R. China

[2] Department of Epidemiology, Third Military Medical University, Chongqing 400038, P.R. China

[3] The Evidence Based Medicine and Clinic Epidemiology Center, Third Military Medical University, Chongqing, 400038, P.R. China

[4] Chongqing Key Laboratory for Diseases Proteomics, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

[*] To whom requests for reprints should be addressed at the Department of Gastroenterology, Third Military Medical University, Chongqing 400037, China. Phone: 86-023-68754678; E-mail: shimingyang@yahoo.com or the Department of Epidemiology, Third Military Medical University, Chongqing 400038, P. R. China sydzy2003@yahoo.com.cn

Running Title: MIF -173 G/C polymorphism and the risk of IBD

Abbreviations: MIF, macrophage migration inhibitory factor; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; OR, odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

Key Words: Crohn's disease, gene polymorphism, inflammatory bowel disease,

macrophage migration inhibitory factor, ulcerative colitis.

Abstract

Objective: To determine whether macrophage migration inhibitory factor (MIF) gene polymorphism is associated with the risk of inflammatory bowel disease (IBD).

Design: System review and meta-analysis.

Methods: MEDLINE, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical Literature database (CBM) were searched for the case-control trails for MIF and IBD. All the studies included into this manuscript met the inclusion and exclusion criteria. An odds ratio (OR) analysis using a 95% confidence interval (CI) was employed to assess the association of the MIF-173 G/C polymorphism with IBD susceptibility.

Results: There was a significant association between the MIF-173 G/C gene polymorphism and IBD in the total population under both the recessive model (CC vs. GC+GG) (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and the codominant model (CC vs. GG) (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). In the stratified analysis by ethnicity, significantly increased risks were observed for Asians using the recessive (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and codominant models (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). Within the subgroups of UC and CD, significant differences were observed regarding UC using the recessive (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and codominant models (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). In the stratified analysis by ethnicity for UC, significant differences were observed regarding CC in Asians vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity).

Conclusion: The meta-analysis suggested that the MIF-173 G/C polymorphism contributed to the susceptibility of IBD. When considering the subgroups of ethnicity and UC and CD, the results suggested that the polymorphism is more significant for UC in Asians.

Article Summary

Article Focus:

1. Examines whether MIF gene polymorphism is associated with IBD, UC or CD.
2. Do Asians and Europeans have different gene mutation points?
3. Are UC and CD caused by the same gene mutation points?

Key messages:

1. It was demonstrated that MIF gene polymorphism is associated with IBD.
2. In Asia, it was found that MIF gene polymorphism is associated with IBD, especially for UC. However, in European, no association was found.
3. In Asia, the main IBD patients were UC, while in European the main patients were CD.

Strengths and limitations of this study:

1. A large number of patients were concluded in this manuscript, which is important when examining the association between MIF and IBD.
2. All the articles included in this manuscript must conform that all controls was in Hardy–Weinberg equilibrium.
3. It still needs to demonstrate that MIF gene polymorphism was associated with IBD in other places such as Africa and South and North America.

Introduction

Inflammatory bowel disease (IBD) currently represents one of the most common health problems worldwide. The highest incidence rates and prevalence of ulcerative colitis (UC) and Crohn's disease (CD) have been reported in northern Europe, the UK, and North America [1]. In addition, in low-incidence areas, such as Asia, southern Europe, and most developing countries, rates also continue to rise [2]. However, the etiology and pathogenesis are still unknown.

Our current understanding of IBD is a complex disease with a number of contributing factors, such as genetic predisposition, environmental factors, intestinal microbial flora, and an aberrant immune response [1]. It has been demonstrated that many IBD patients have a dysregulated intestinal mucosal T-cell-mediated immune response, specifically involving CD4 T helper type-1 (Th1) lymphocytes, which leads to the production of Th1-associated pro-inflammatory cytokines, such as interferon-γ (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-alpha (TNF-α)[3].

In addition, another cytokine, macrophage migration inhibitory factor (MIF), is considered to play a critical role in immunity and inflammation. MIF is secreted by a series of immune cells, such as macrophages, dendritic cells, lymphocytes, neutrophils and pituitary cells[4 5]. Once secreted, MIF regulates a broad range of immune and inflammatory activities, including the induction of inflammatory cytokines, such as TNF-α, IFN-γ, IL-1β, IL-12, IL-6 and, CXCL8 (also known as IL-8), among others [6]. However, if the MIF gene is mutated, it will disturb the immune balance in the microenvironment. It has been demonstrated that MIF gene

mutation is associated with many autoimmune diseases, such as rheumatoid arthritis, glomerulonephritis, and inflammatory bowel diseases [7-11]. MIF genotyping studies have focused on the -794 $CATT_{(5–8)}$ microsatellite and the -173 G/C polymorphism[12]. Donn et al first reported that the MIF 173 polymorphism is a risk factor for juvenile idiopathic arthritis [13]. Consequently, Baugh et al reported the association between the -794 $CATT_{(5–8)}$ microsatellite and disease severity in patients with rheumatoid arthritis[14]. In addition, it has also been reported that the MIF gene polymorphism is a risk factor for other immune diseases, such as atrophy, asthma and sarcoidosis in erythema nodosum patients[12]. Therefore, it is important to explore whether MIF gene polymorphisms are associated with IBD. However, the research results are not in agreement. Thomas and colleagues found that there was no significant difference between IBD patients and controls with the MIF gene type [15]. In other articles, the MIF gene polymorphism has been reported to be a risk factor for IBD [16 17]. Because most of the articles on MIF gene polymorphisms and IBD studied the -173 G/C polymorphism, we performed a meta-analysis to determine whether the MIF -173 G/C polymorphism is a risk factor for IBD. Our analysis reveals that the MIF -173 G/C polymorphism is a risk factor for IBD, especially for UC in Asians.

Materials and Methods

Search Strategy

This meta-analysis followed the proposal of the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group and was performed by searching PubMed, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical

Literature database (CBM) (last search updated in April, 2013) [18]. The search strategy included the following terms: (macrophage migration inhibitory factor [MeSH] or MIF [TEXT WORD] and inflammatory bowel disease [MeSH] or ulcerative colitis [TEXT WORD] or Crohn's disease [TEXT WORD] or IBD [TEXT WORD] or UC [TEXT WORD] or CD [TEXT WORD]). Searches also included scanning reference lists in relevant articles and conference proceedings as well as correspondence with authors when additional data were required. Two reviewers (Hao and He) independently screened the titles and abstracts of each identified reference and categorized papers based on the full text to evaluate their eligibility for inclusion.

Inclusion and Exclusion Criteria

The inclusion criteria for primary studies were as follows: (1) the article evaluated MIF gene polymorphisms and IBD risk; (2) the article included case–control studies or a nested case–control study; (3) the article supplied the number of individual genotypes for the MIF polymorphisms in inflammatory bowel disease cases and controls; and (4) the article demonstrated that the distribution of genotypes among controls was in Hardy–Weinberg equilibrium. In addition, the following exclusion criteria were used. the studies were excluded when 1) it did not provide detailed data such as presented in abstracts, meeting reports and reviews; 2) the studies were repeated or overlapped other publications; 3) the genotype frequency was not reported; and 4) the controls do not meet the assumptions for HWE.

Data Extraction

No paper was included if it did not meet the four inclusion criteria. When the same study results appeared in several papers, only one study was used in this meta-analysis. Table 1 lists the characteristics of the extracted data, including the first author's name, publication date, region of study, ethnicity of the sample population, number of genotypes, and the total number of cases and controls. The study regions included China, Japan, Spain, Germany and Poland. Data extraction was independently performed by two individuals (Hao and He), and any disagreement was resolved by consensus or by consultation with additional reviewers (Zhang and Yang).

Qualitative Assessment

Quality assessment was performed with the Newcastle-Ottawa quality assessment scale (NOS) for case-control studies. A 'star system' was used to judge data quality based on three broad perspectives: selection, comparability and outcome of interest for case-control studies. Star counts were totaled to compare the study quality in a quantitative fashion[19]. Based on these criteria, the content validity was evaluated by Hao and He, and any disagreement was resolved via discussions between Hao and Luo or with the other authors (Zhang and Yang) for adjudication.

Statistical Analysis

All statistical tests were performed using Revman 5.0 software. Deviations from Hardy–Weinberg equilibrium (HWE) were calculated for the control groups by χ^2 goodness-of-fit. The association between the MIF -173G/C gene polymorphism and IBD was compared by the odds ratio (OR) and the corresponding 95% confidence

interval (CI) between the case and control groups. The statistical significance of the summary OR was determined with the Z test, and P less than 0.05 was considered as statistically significant. The genetic models evaluated for the polymorphism were the dominant model (GC+CC vs. GG), the recessive model (CC vs. GC+GG), the allelic gene model (C vs. G) and the codominant model (CC vs. GG and GC vs. GG).

The heterogeneity between studies was determined by the Chi-square-based Q-test. A P value greater than 0.05 for the Q-test indicated a lack of heterogeneity among the studies. If there was a lack of heterogeneity, the pooled OR estimate of each study was calculated using a fixed-effects model (the Mantel–Haenszel method); otherwise, a random-effects model (the DerSimonian and Laird method) was used [20 21]. In addition, subgroup analysis stratified by ethnicity and different disease types was also performed. The potential publication bias was estimated by a funnel plot. Egger's linear regression test on the natural logarithm scale of the OR was used to assess the funnel plot asymmetry; the significance was set at the P<0.05 level [22].

Results

Study Characteristics

Figure 1 outlines our search process. First, a total of 123 articles were collected after the initial search with the key words listed previously. After reading the title and abstract, 12 reviews and 1 editorial were excluded. Next, 99 articles were excluded for no association with IBD and the MIF -173 G/C polymorphism. The remaining 11 articles were identified for full-text review, and 4 articles were excluded because of repeated publications. In addition, 1 article was excluded because it deviated from

HWE [23]. Finally, 6 articles met our inclusion criteria and were used for the meta-analysis with 2084 cases and 2288 controls [15-17 24-26]. The basic characteristics of these articles are listed in Table 1. Of the eligible studies, 3/6 articles researched the association of the MIF-173 gene polymorphism with both UC and CD. The remaining 3/6 articles only researched the association of the MIF -173 G/C polymorphism with UC. In total, 3/6 studies were performed in Europeans, and 3/6 were performed in Asians. All studies were cross-sectional case-control studies with sufficient data to calculate the possible relationship between the MIF -173 G/C polymorphism and IBD. All studies were published in English. The distribution of the MIF -173 gene type in IBD, UC and CD is listed in Table 2.

Quantitative Data Synthesis

Table 3 lists the primary results. In the total population, we found a significant difference between the MIF -173 G/C gene polymorphism and the risk of IBD for two variants: CC vs. GC+GG (OR=1.5, CI =1.07-2.14, P=0.02 for heterogeneity) and CC vs. GG (OR=1.54, CI=1.09-2.24, P=0.01 for heterogeneity) (Figures 2 A， B， C and D). No significant difference was observed for the variants of GC+CC vs. GG, GC vs. GG or the allele C vs. G (Table 3).

In the stratified analysis by ethnicity, a significantly increased risk was observed in Asians for CC vs. GC+GG (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and CC vs. GG (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). (Figure 2 A, B) However, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.36, CI=0.86-2.15, P=0.19 for heterogeneity) or CC vs. GG (OR=1.42,

CI=0.89-2.24, P=0.14 for heterogeneity).

Because IBD includes UC and CD, we determined whether the role of the MIF -173 G/C gene polymorphism was different between the two diseases. Therefore, we analyzed subgroups of UC and CD. Significant differences were observed in UC for CC vs. GC+GG (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and CC vs. GG (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). (Figure 2 C and D) However, no significant differences were found in CD for CC vs. GC+GG (OR=1.41, CI=0.85-2.36, P=0.19 for heterogeneity) or CC vs. GG (OR=1.44, CI=0.86-2.40, P=0.16 for heterogeneity).

Although IBD includes UC and CD, the cases of IBD in Asians included in our data were mainly UC, and only 15 cases were CD. This may influence the accuracy of the meta-analysis results in the subgroup of ethnicity. Therefore, we analyzed the association of the MIF -173 G/C polymorphism with UC in the subgroup of ethnicity. As shown in Figure 3A, a significant difference was observed in Asians for CC vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity). Similar to previous results, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.47, CI=0.85-2.55, P=0.17 for heterogeneity).

Sensitivity Analyses and Publication Bias

Sensitivity analyses were performed to assess the influence of each individual study on the pooled ORs by the systematic omission of the individual studies from the analyses. the corresponding pooled OR was not materially altered. Begg's funnel plot and the Egger's test were performed to assess the publication bias of the literature. As

shown in Figure 3B, the shape of the funnel plot did not reveal obvious asymmetry. The Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias (data not shown).

Discussion

The human MIF gene, which is located on chromosome 22q11.2, is short; it is composed of 3 exons of 205, 173, and 183 bp and 2 introns of 189 and 95 bp [12 27 28]. Four polymorphisms of the human MIF gene have been reported, including a 5–8 CATT tetranucleotide repeat at position -794 $CATT_{(5–8)}$ and 3 single-nucleotide polymorphisms (SNPs) at positions -173 G/C, +254 T/C, and +656 C/G [13 14 29]. However, in IBD, studies on MIF gene polymorphisms have mainly focused on the -173 G/C SNP. Therefore, in this meta-analysis, we mainly discussed the association of the MIF -173 G/C gene polymorphism with the susceptibility to IBD.

In the current meta-analysis of 6 studies consisting of 2084 cases and 2288 controls, we found that the MIF -173 G/C gene polymorphism is significantly associated with IBD susceptibility in both the recessive model (OR: 1.5) and codominant model (OR: 1.54), while no significant associations were found in the dominant model or the allelic model. The results indicated that the MIF -173 G/C polymorphism was a conspicuous high-risk factor for developing IBD in the overall study population.

The second finding of this meta-analysis is that in the subgroup of ethnicity, the MIF -173 G/C gene polymorphism was significantly different in Asians in the recessive (OR: 1.75) and codominant (OR: 1.74) models, while no significant

differences were found in Europeans. This finding is consistent with previous results that a gene polymorphism does not have the same effect in different ethnicities. For example, the TNF-α308A gene polymorphism plays an important role in Asian populations, while no conclusive data on this association exist in European patients [30]. In addition, the best studied genetic variant, a nucleotide oligomerization domain (NOD)2 polymorphism, is present in up to 20% of patients with CD in White and Jewish populations, but major disease-associated variants have not been detected in individuals of Asian descent with CD [31 32].

The third finding of this meta-analysis is that in the subgroup of the two diseases, UC and CD, significant differences were found in the recessive (OR: 1.60) and codominant (OR: 1.64) model for UC, while no difference was found in the recessive or codominant model for CD. This result suggests that the MIF -173 G/C polymorphism seems to be a risk factor for UC but not for CD. However, in another study, Zhang and colleagues confirmed that the peroxisome proliferator-activated receptor gamma (PPARγ) AlaAla genotype is a protective factor against the development of CD, especially in the European Caucasian population, while no significant association was found in the development of UC [33]. Both of these results indicated that the gene polymorphism has different effects in different diseases.

To further determine whether the MIF -173 gene polymorphism for UC was different in different ethnicities, we performed a meta-analysis of UC in the subgroup of ethnicity. A significant difference was found in the recessive model (OR: 1.73) in Asians, while no significant difference was found in Europeans. Although only three

studies were included in the sub-group analysis, they all have high quality. So the results in the sub-group analysis were credible.

In addition, two similar studies had been published. Compared to the two studies 34 35, our study has the strength. First it has been claimed that the studies included in the meta-analysis should not deviated from HWE. So in our study we didn't include the study from India.23 Second, in our study, we analysis MIF polymorphism and the risk of UC，since the number of CD cases in Asia were low, which may made the bias of MIF polymorphism and IBD risk. However, the present meta-analysis had several limitations that must be taken into consideration. First, the number of available studies that could be included in this meta-analysis was moderate. Therefore, the results could be influenced by factors such as random error. Therefore, next we will focus on research on the association between gene polymorphisms and IBD in our hospitals. Second, the overall outcomes were based on individual unadjusted ORs, while a more precise evaluation should be adjusted by other potentially suspected factors, including age, sex, and environmental factors.

In summary, the current meta-analysis suggested that the MIF -173 G/C polymorphism contributed to the susceptibility of IBD. In the subgroups of ethnicity and UC and CD, the result suggested that this polymorphism was more significant for UC in Asians.

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Author contributorship statement

1. Hao,and He contributed to conception and design, acquisition of data, or analysis and interpretation of data.
2. Hao and Yong acquisition of data
3. Hao, He, Luo and Zhang analyses and interpreted the data.
4. Hao and Yang drafted the article.
5. All the authors listed in this manuscript approval to publish in ***BMJ OPEN***.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data Sharing Statement

No additional data

Reference

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Macrophage migration inhibitory factor polymorphism and the risk of Ulcerative Colitis and Crohn's Disease in Asian and European: A meta-analysis

Ning-Bo Hao[1], Ya Fei He[1], Gang Luo[1], Xin Yong[1], Yao Zhang[2, 3,*] and Shi-Ming Yang[1, 4, *]

[1] Department of Gastroenterology, Xinqiao Hospital, Third Military Medical University; Chongqing 400038, P.R. China

[2] Department of Epidemiology, Third Military Medical University, Chongqing 400038, P.R. China

[3] The Evidence Based Medicine and Clinic Epidemiology Center, Third Military Medical University, Chongqing, 400038, P.R. China

[4] Chongqing Key Laboratory for Diseases Proteomics, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

[*] To whom requests for reprints should be addressed at the Department of Gastroenterology, Third Military Medical University, Chongqing 400037, China. Phone: 86-023-68754678; E-mail: shimingyang@yahoo.com or the Department of Epidemiology, Third Military Medical University, Chongqing 400038, P. R. China sydzy2003@yahoo.com.cn

Running Title: MIF -173 G/C polymorphism and the risk of IBD

Abbreviations: MIF, macrophage migration inhibitory factor; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; OR, odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

Abstract

Objective: To determine whether macrophage migration inhibitory factor (MIF) gene polymorphism is associated with the risk of inflammatory bowel disease (IBD).

Design: System review and meta-analysis.

Methods: MEDLINE, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical Literature database (CBM) were searched for the case-control trails for MIF and IBD. All the studies included into this manuscript met the inclusion and exclusion criteria. An odds ratio (OR) analysis using a 95% confidence interval (CI) was employed to assess the association of the MIF-173 G/C polymorphism with IBD susceptibility.

Results: There was a significant association between the MIF-173 G/C gene polymorphism and IBD in the total population under both the recessive model (CC vs. GC+GG) (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and the codominant model (CC vs. GG) (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). In the stratified analysis by ethnicity, significantly increased risks were observed for Asians using the recessive (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and codominant models (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). Within the subgroups of UC and CD, significant differences were observed regarding UC using the recessive (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and codominant models (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). In the stratified analysis by ethnicity for UC, significant differences were observed regarding CC in Asians vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity).

Conclusion: The meta-analysis suggested that the MIF-173 G/C polymorphism contributed to the susceptibility of IBD. When considering the subgroups of ethnicity and UC and CD, the results suggested that the polymorphism is more significant for UC in Asians.

Key Words: Crohn's disease, gene polymorphism, inflammatory bowel disease, macrophage migration inhibitory factor, ulcerative colitis.

Introduction

Inflammatory bowel disease (IBD) currently represents one of the most common health problems worldwide. The highest incidence rates and prevalence of ulcerative colitis (UC) and Crohn's disease (CD) have been reported in northern Europe, the UK, and North America [1]. In addition, in low-incidence areas, such as Asia, southern Europe, and most developing countries, rates also continue to rise [2]. However, the etiology and pathogenesis are still unknown.

Our current understanding of IBD is a complex disease with a number of contributing factors, such as genetic predisposition, environmental factors, intestinal microbial flora, and an aberrant immune response [1]. It has been demonstrated that many IBD patients have a dysregulated intestinal mucosal T-cell-mediated immune response, specifically involving CD4 T helper type-1 (Th1) lymphocytes, which leads to the production of Th1-associated pro-inflammatory cytokines, such as interferon-γ (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-alpha (TNF-α)[3].

In addition, another cytokine, macrophage migration inhibitory factor (MIF), is considered to play a critical role in immunity and inflammation. MIF is secreted by a series of immune cells, such as macrophages, dendritic cells, lymphocytes, neutrophils and pituitary cells[4 5]. Once secreted, MIF regulates a broad range of immune and inflammatory activities, including the induction of inflammatory cytokines, such as TNF-α, IFN-γ, IL-1β, IL-12, IL-6 and, CXCL8 (also known as IL-8), among others [6]. However, if the MIF gene is mutated, it will disturb the immune balance in the microenvironment. It has been demonstrated that MIF gene

mutation is associated with many autoimmune diseases, such as rheumatoid arthritis, glomerulonephritis, and inflammatory bowel diseases [7-11]. MIF genotyping studies have focused on the -794 $CATT_{(5–8)}$ microsatellite and the -173 G/C polymorphism[12]. Donn et al first reported that the MIF 173 polymorphism is a risk factor for juvenile idiopathic arthritis [13]. Consequently, Baugh et al reported the association between the -794 $CATT_{(5–8)}$ microsatellite and disease severity in patients with rheumatoid arthritis[14]. In addition, it has also been reported that the MIF gene polymorphism is a risk factor for other immune diseases, such as atrophy, asthma and sarcoidosis in erythema nodosum patients[12]. Therefore, it is important to explore whether MIF gene polymorphisms are associated with IBD. However, the research results are not in agreement. Thomas and colleagues found that there was no significant difference between IBD patients and controls with the MIF gene type [15]. In other articles, the MIF gene polymorphism has been reported to be a risk factor for IBD [16 17]. Because most of the articles on MIF gene polymorphisms and IBD studied the -173 G/C polymorphism, we performed a meta-analysis to determine whether the MIF -173 G/C polymorphism is a risk factor for IBD. Our analysis reveals that the MIF -173 G/C polymorphism is a risk factor for IBD, especially for UC in Asians.

Materials and Methods

Search Strategy

This meta-analysis followed the proposal of the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group and was performed by searching PubMed, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical

Literature database (CBM) (last search updated in April, 2013) [18]. The search strategy included the following terms: (macrophage migration inhibitory factor [MeSH] or MIF [TEXT WORD] and inflammatory bowel disease [MeSH] or ulcerative colitis [TEXT WORD] or Crohn's disease [TEXT WORD] or IBD [TEXT WORD] or UC [TEXT WORD] or CD [TEXT WORD]). Searches also included scanning reference lists in relevant articles and conference proceedings as well as correspondence with authors when additional data were required. Two reviewers (Hao and He) independently screened the titles and abstracts of each identified reference and categorized papers based on the full text to evaluate their eligibility for inclusion.

Inclusion and Exclusion Criteria

The inclusion criteria for primary studies were as follows: (1) the article evaluated MIF gene polymorphisms and IBD risk; (2) the article included case–control studies or a nested case–control study; (3) the article supplied the number of individual genotypes for the MIF polymorphisms in inflammatory bowel disease cases and controls; and (4) the article demonstrated that the distribution of genotypes among controls was in Hardy–Weinberg equilibrium. In addition, the following exclusion criteria were used. the studies were excluded when 1) it did not provide detailed data such as presented in abstracts, meeting reports and reviews; 2) the studies were repeated or overlapped other publications; 3) the genotype frequency was not reported; and 4) the controls do not meet the assumptions for HWE.

Data Extraction

No paper was included if it did not meet the four inclusion criteria. When the

same study results appeared in several papers, only one study was used in this meta-analysis. Table 1 lists the characteristics of the extracted data, including the first author's name, publication date, region of study, ethnicity of the sample population, number of genotypes, and the total number of cases and controls. The study regions included China, Japan, Spain, Germany and Poland. Data extraction was independently performed by two individuals (Hao and He), and any disagreement was resolved by consensus or by consultation with additional reviewers (Zhang and Yang).

Qualitative Assessment

Quality assessment was performed with the Newcastle-Ottawa quality assessment scale (NOS) for case-control studies. A 'star system' was used to judge data quality based on three broad perspectives: selection, comparability and outcome of interest for case-control studies. Star counts were totaled to compare the study quality in a quantitative fashion[19]. Based on these criteria, the content validity was evaluated by Hao and He, and any disagreement was resolved via discussions between Hao and Luo or with the other authors (Zhang and Yang) for adjudication.

Statistical Analysis

All statistical tests were performed using Revman 5.0 software. Deviations from Hardy–Weinberg equilibrium (HWE) were calculated for the control groups by χ^2 goodness-of-fit. The association between the MIF -173G/C gene polymorphism and IBD was compared by the odds ratio (OR) and the corresponding 95% confidence interval (CI) between the case and control groups. The statistical significance of the summary OR was determined with the Z test, and P less than 0.05 was considered as

statistically significant. The genetic models evaluated for the polymorphism were the dominant model (GC+CC vs. GG), the recessive model (CC vs. GC+GG), the allelic gene model (C vs. G) and the codominant model (CC vs. GG and GC vs. GG).

The heterogeneity between studies was determined by the Chi-square-based Q-test. A P value greater than 0.05 for the Q-test indicated a lack of heterogeneity among the studies. If there was a lack of heterogeneity, the pooled OR estimate of each study was calculated using a fixed-effects model (the Mantel–Haenszel method); otherwise, a random-effects model (the DerSimonian and Laird method) was used [20 21]. In addition, subgroup analysis stratified by ethnicity and different disease types was also performed. The potential publication bias was estimated by a funnel plot. Egger's linear regression test on the natural logarithm scale of the OR was used to assess the funnel plot asymmetry; the significance was set at the $P<0.05$ level [22].

Results

Study Characteristics

Figure 1 outlines our search process. First, a total of 123 articles were collected after the initial search with the key words listed previously. After reading the title and abstract, 12 reviews and 1 editorial were excluded. Next, 99 articles were excluded for no association with IBD and the MIF -173 G/C polymorphism. The remaining 11 articles were identified for full-text review, and 4 articles were excluded because of repeated publications. In addition, 1 article was excluded because it deviated from HWE [23]. Finally, 6 articles met our inclusion criteria and were used for the meta-analysis with 2084 cases and 2288 controls [15-17 24-26]. The basic characteristics of

these articles are listed in Table 1. Of the eligible studies, 3/6 articles researched the association of the MIF-173 gene polymorphism with both UC and CD. The remaining 3/6 articles only researched the association of the MIF -173 G/C polymorphism with UC. In total, 3/6 studies were performed in Europeans, and 3/6 were performed in Asians. All studies were cross-sectional case-control studies with sufficient data to calculate the possible relationship between the MIF -173 G/C polymorphism and IBD. All studies were published in English. The distribution of the MIF -173 gene type in IBD, UC and CD is listed in Table 2.

Quantitative Data Synthesis

Table 3 lists the primary results. In the total population, we found a significant difference between the MIF -173 G/C gene polymorphism and the risk of IBD for two variants: CC vs. GC+GG (OR=1.5, CI =1.07-2.14, P=0.02 for heterogeneity) and CC vs. GG (OR=1.54, CI=1.09-2.24, P=0.01 for heterogeneity) (Figures 2 A， B， C and D). No significant difference was observed for the variants of GC+CC vs. GG, GC vs. GG or the allele C vs. G (Table 3).

In the stratified analysis by ethnicity, a significantly increased risk was observed in Asians for CC vs. GC+GG (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and CC vs. GG (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). (Figure 2 A, B) However, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.36, CI=0.86-2.15, P=0.19 for heterogeneity) or CC vs. GG (OR=1.42, CI=0.89-2.24, P=0.14 for heterogeneity).

Because IBD includes UC and CD, we determined whether the role of the MIF

-173 G/C gene polymorphism was different between the two diseases. Therefore, we analyzed subgroups of UC and CD. Significant differences were observed in UC for CC vs. GC+GG (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and CC vs. GG (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). (Figure 2 C and D) However, no significant differences were found in CD for CC vs. GC+GG (OR=1.41, CI=0.85-2.36, P=0.19 for heterogeneity) or CC vs. GG (OR=1.44, CI=0.86-2.40, P=0.16 for heterogeneity).

Although IBD includes UC and CD, the cases of IBD in Asians included in our data were mainly UC, and only 15 cases were CD. This may influence the accuracy of the meta-analysis results in the subgroup of ethnicity. Therefore, we analyzed the association of the MIF -173 G/C polymorphism with UC in the subgroup of ethnicity. As shown in Figure 3A, a significant difference was observed in Asians for CC vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity). Similar to previous results, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.47, CI=0.85-2.55, P=0.17 for heterogeneity).

Sensitivity Analyses and Publication Bias

Sensitivity analyses were performed to assess the influence of each individual study on the pooled ORs by the systematic omission of the individual studies from the analyses. the corresponding pooled OR was not materially altered. Begg's funnel plot and the Egger's test were performed to assess the publication bias of the literature. As shown in Figure 3B, the shape of the funnel plot did not reveal obvious asymmetry. The Egger's test was used to provide statistical evidence of funnel plot symmetry. The

results still did not suggest any evidence of publication bias (data not shown).

Discussion

The human MIF gene, which is located on chromosome 22q11.2, is short; it is composed of 3 exons of 205, 173, and 183 bp and 2 introns of 189 and 95 bp [12 27 28]. Four polymorphisms of the human MIF gene have been reported, including a 5–8 CATT tetranucleotide repeat at position -794 $CATT_{(5–8)}$ and 3 single-nucleotide polymorphisms (SNPs) at positions -173 G/C, +254 T/C, and +656 C/G [13 14 29]. However, in IBD, studies on MIF gene polymorphisms have mainly focused on the -173 G/C SNP. Therefore, in this meta-analysis, we mainly discussed the association of the MIF -173 G/C gene polymorphism with the susceptibility to IBD.

In the current meta-analysis of 6 studies consisting of 2084 cases and 2288 controls, we found that the MIF -173 G/C gene polymorphism is significantly associated with IBD susceptibility in both the recessive model (OR: 1.5) and codominant model (OR: 1.54), while no significant associations were found in the dominant model or the allelic model. The results indicated that the MIF -173 G/C polymorphism was a conspicuous high-risk factor for developing IBD in the overall study population.

The second finding of this meta-analysis is that in the subgroup of ethnicity, the MIF -173 G/C gene polymorphism was significantly different in Asians in the recessive (OR: 1.75) and codominant (OR: 1.74) models, while no significant differences were found in Europeans. This finding is consistent with previous results that a gene polymorphism does not have the same effect in different ethnicities. For

example, the TNF-α308A gene polymorphism plays an important role in Asian populations, while no conclusive data on this association exist in European patients [30]. In addition, the best studied genetic variant, a nucleotide oligomerization domain (NOD)2 polymorphism, is present in up to 20% of patients with CD in White and Jewish populations, but major disease-associated variants have not been detected in individuals of Asian descent with CD [31 32].

The third finding of this meta-analysis is that in the subgroup of the two diseases, UC and CD, significant differences were found in the recessive (OR: 1.60) and codominant (OR: 1.64) model for UC, while no difference was found in the recessive or codominant model for CD. This result suggests that the MIF -173 G/C polymorphism seems to be a risk factor for UC but not for CD. However, in another study, Zhang and colleagues confirmed that the peroxisome proliferator-activated receptor gamma (PPARγ) AlaAla genotype is a protective factor against the development of CD, especially in the European Caucasian population, while no significant association was found in the development of UC [33]. Both of these results indicated that the gene polymorphism has different effects in different diseases.

To further determine whether the MIF -173 gene polymorphism for UC was different in different ethnicities, we performed a meta-analysis of UC in the subgroup of ethnicity. A significant difference was found in the recessive model (OR: 1.73) in Asians, while no significant difference was found in Europeans. Although only three studies were included in the sub-group analysis, they all have high quality. So the results in the sub-group analysis were credible.

In addition, two similar studies had been published. Compared to the two studies 34 35, our study has the strength. First it has been claimed that the studies included in the meta-analysis should not deviated from HWE. So in our study we didn't include the study from India.23 Second, in our study, we analysis MIF polymorphism and the risk of UC，since the number of CD cases in Asia were low, which may made the bias of MIF polymorphism and IBD risk. However, the present meta-analysis had several limitations that must be taken into consideration. First, the number of available studies that could be included in this meta-analysis was moderate. Therefore, the results could be influenced by factors such as random error. Therefore, next we will focus on research on the association between gene polymorphisms and IBD in our hospitals. Second, the overall outcomes were based on individual unadjusted ORs, while a more precise evaluation should be adjusted by other potentially suspected factors, including age, sex, and environmental factors.

In summary, the current meta-analysis suggested that the MIF -173 G/C polymorphism contributed to the susceptibility of IBD. In the subgroups of ethnicity and UC and CD, the result suggested that this polymorphism was more significant for UC in Asians.

Acknowledgements

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Conflict of interest

The authors declare that they have no conflict of interest.

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Article Summary

Article Focus:

1. Examines whether MIF gene polymorphism is associated with IBD, UC or CD.
2. Do Asians and Europeans have different gene mutation points?
3. Are UC and CD caused by the same gene mutation points?

Key messages:

1. It was demonstrated that MIF gene polymorphism is associated with IBD.
2. In Asia, it was found that MIF gene polymorphism is associated with IBD, especially for UC. However, in European, no association was found.
3. In Asia, the main IBD patients were UC, while in European the main patients were CD.

Strengths and limitations of this study:

1. A large number of patients were concluded in this manuscript, which is important when examining the association between MIF and IBD.
2. All the articles included in this manuscript must conform that all controls was

in Hardy–Weinberg equilibrium.

3. It still needs to demonstrate that MIF gene polymorphism was associated with IBD in other places such as Africa and South and North America.

Author contributorship statement

1. Hao,and He contributed to conception and design, acquisition of data, or analysis and interpretation of data.
2. Hao and Yong acquisition of data
3. Hao, He, Luo and Zhang analyses and interpreted the data.
4. Hao and Yang drafted the article.
5. All the authors listed in this manuscript approval to publish in ***BMJ OPEN***.

Table1 Characteristics of studies included in meta-analysis

First author	Years	Country	Ethnicity	Case number(n)	Control Number (n)	Newcastle-Ottawa Score	Genotyping
Griaga	2007	Germany	European	259	489	7/9	PCR-RFLP
Shiroeda	2010	Japan	Asian	111	209	6/9	PCR-SSCP
Fei	2008	China	Asian	99	142	9/9	PCR-RFLP tetra-primer ARMS
Oliver	2007	Spain	European	1295	887	8/9	PCR-RFLP
Przybyłowska	2011	Poland	European	99	123	6/9	PCR-RFLP
Nohara	2004	Japan	Asian	221	438	7/9	PCR-RFLP PCR-SSCP

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism， ARMS： amplification refractory mutation system

PCR-SSCP, polymerase chain reaction-single-strand conformation polymorphism，

Table 2: Genotypes and allele frequencies of MIF-173G/C genes in patients and controls

Author	Year	Case					Control					Sample size	HWE（P）
		GG	GC	CC	G	C	GG	GC	CC	G	C		
IBD													
Fei	2008	52	32	15	136	62	79	55	8	213	71	99/142	0.70
Griaga	2007	188	67	4	443	75	318	156	15	792	186	259/489	0.43
Nohara	2004	135	76	10	346	96	288	134	16	710	166	221/438	0.93
Shiroeda	2010	69	37	5	175	47	126	76	7	328	90	111/209	0.27
Przybyłowska	2011	66	31	2	163	35	99	23	1	221	25	99/123	0.79
Oliver	2007	907	343	45	2157	433	681	188	18	1550	224	1295/887	0.24
UC													
Fei	2008	44	27	13	115	53	79	55	8	213	71	84/142	0.70
Griaga	2007	72	28	2	172	32	318	156	15	792	186	102/489	0.43
Nohara	2004	135	76	10	346	96	288	134	16	710	166	221/438	0.93
Oliver	2007	441	171	22	1053	215	681	188	18	1550	224	634/887	0.24

Przybyłowska	2011	38	19	1	95	21	99	23	1	221	25	58/123	0.79
Shiroeda	2010	69	37	5	175	47	126	76	7	328	90	111/209	0.27
CD													
Fei	2008	8	5	2	21	9	79	55	8	213	71	15/142	0.70
Griaga	2007	116	39	2	271	43	318	156	15	792	186	157/489	0.43
Oliver	2007	466	172	23	1104	218	681	188	18	1550	224	661/887	0.24
Przybyłowska	2011	28	12	1	68	14	99	23	1	221	25	41/123	0.79

HWE: Hardy-Weinberg Equilibrium

Table 3 Summary of different comparative results

	Study	Sample size		No. of	Test of association			Model	Heterogeneity		
		case	control	studies	OR (95% CI)	*Z*	*P* Value		χ^2	*P* Value	I^2 (%)
GC+CC vs. GG	Overall	2084	2288	6	1.15（0.87-1.52）	0.97	0.33	R	17.04	0.004	71.0
	Asian	431	789	3	1.12 (0.88 – 1.44)	0.90	0.37	F	0.90	0.64	0.0
	European	1653	1499	3	1.23 (0.70 – 2.14)	0.71	0.48	R	15.75	0.0004	87.0
	UC	1210	2288	6	1.24 (1.06 – 1.44)	2.76	0.006	F	9.69	0.08	48.0
	CD	874	1641	4	1.13 (0.69 – 1.87)	0.49	0.62	F	11.46	0.009	74.0
CC vs. GC+GG	Overall	2084	2282	6	1.51(1.07-2.14)	2.34	0.02	F	6.72	0.24	26.0
	Asian	431	789	3	1.75(1.04 – 2.95)	2.10	0.04	F	2.21	0.33	9.0
	European	1653	1499	3	1.36 (0.86 – 2.15)	1.31	0.19	F	4.16	0.12	52.0
	UC	1210	2288	6	1.60 (1.09 – 2.35)	2.42	0.02	F	3.92	0.56	0.0
	CD	874	1641	4	1.41 (0.85 – 2.36)	1.32	0.19	F	3.91	0.27	23.0
CC vs. GG	Overall	1498	1656	6	1.54（1.09-2.24）	2.43	0.01	F	7.30	0.20	32.0
	Asian	286	524	3	1.74（1.02– 2.97）	2.04	0.04	F	1.72	0.42	0.0
	European	1212	1132	3	1.42 (0.89 – 2.24)	1.49	0.14	F	5.38	0.07	63.0
	UC	852	1656	6	1.64 (1.12 – 2.41)	2.52	0.01	F	3.88	0.57	0.0
	CD	646	1219	4	1.44 (0.86 – 2.40)	1.40	0.16	F	4.68	0.20	36.0
GC vs. GG	Overall	2003	1972	6	1.40（1.00-1.95)	1.97	0.05	R	18.46	0.002	73.0
	Asian	401	810	3	1.02 (0.79 – 1.31)	0.14	0.89	F	1.01	0.60	0.0
	European	1602	1465	3	1.21 (0.72 – 2.03)	0.73	0.47	R	12.77	0.002	84.0
	UC	1157	2223	6	1.20 (1.02 – 1.40)	2.20	0.03	F	9.64	0.09	48.0
	CD	846	1599	4	1.09 (0.69 – 1.74)	0.39	0.70	R	9.13	0.03	67.0
C vs. G	Overall	4168	4576	6	1.17 (0.91 – 1.50)	1.25	0.21	R	18.47	0.002	73.0
	Asian	862	1578	3	1.17 (0.96 – 1.43)	1.54	0.12	F	1.36	0.51	0.0
	European	3306	2998	3	1.20 (0.72 – 2.01)	0.70	0.49	R	17.04	0.0002	88.0
	UC	2420	4576	6	1.24 (1.09 – 1.41)	3.20	0.001	F	9.51	0.08	50.0
	CD	1748	3282	4	1.16(0.73 – 1.83)	0.63	0.53	R	12.78	0.005	77.0

* vs.: versus; R: random-effects model; F: fixed-effects model.

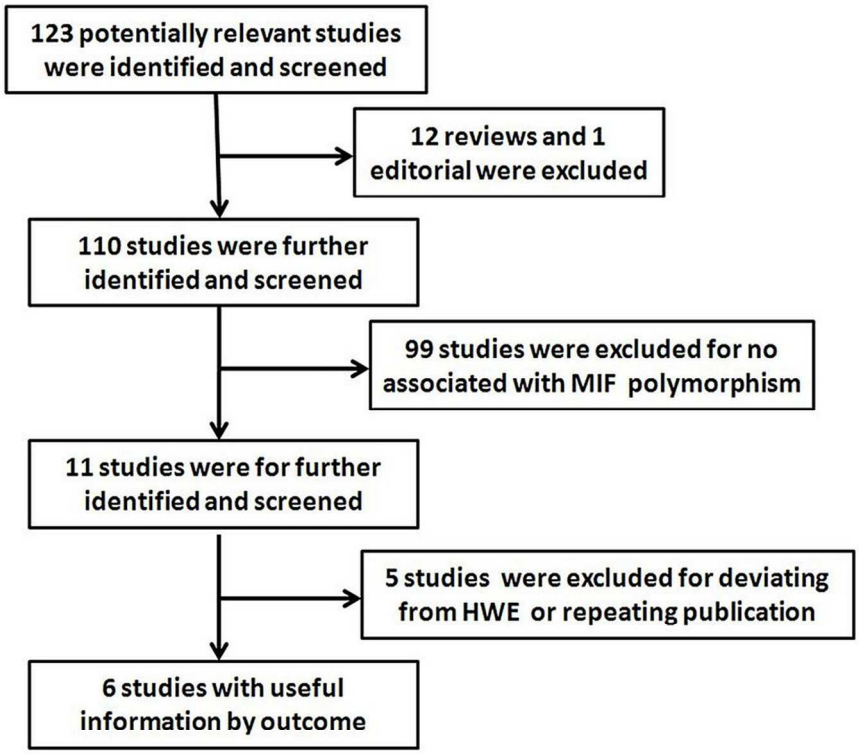
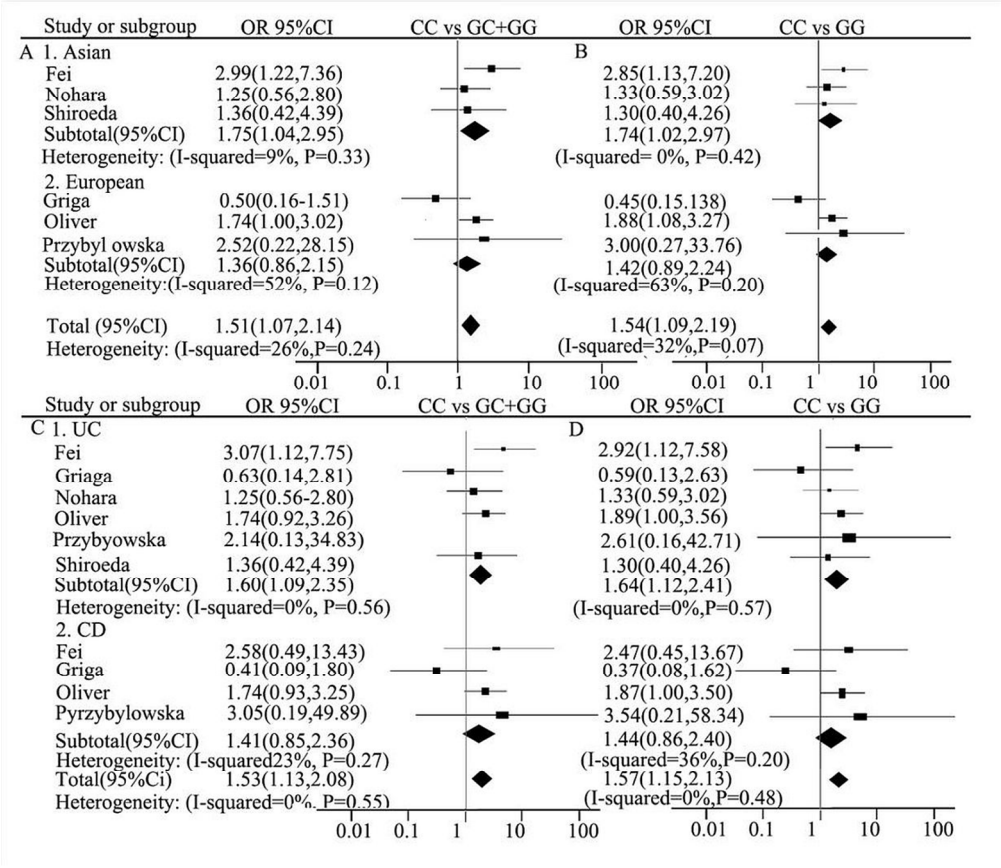


Figure 1

105x90mm (300 x 300 DPI)



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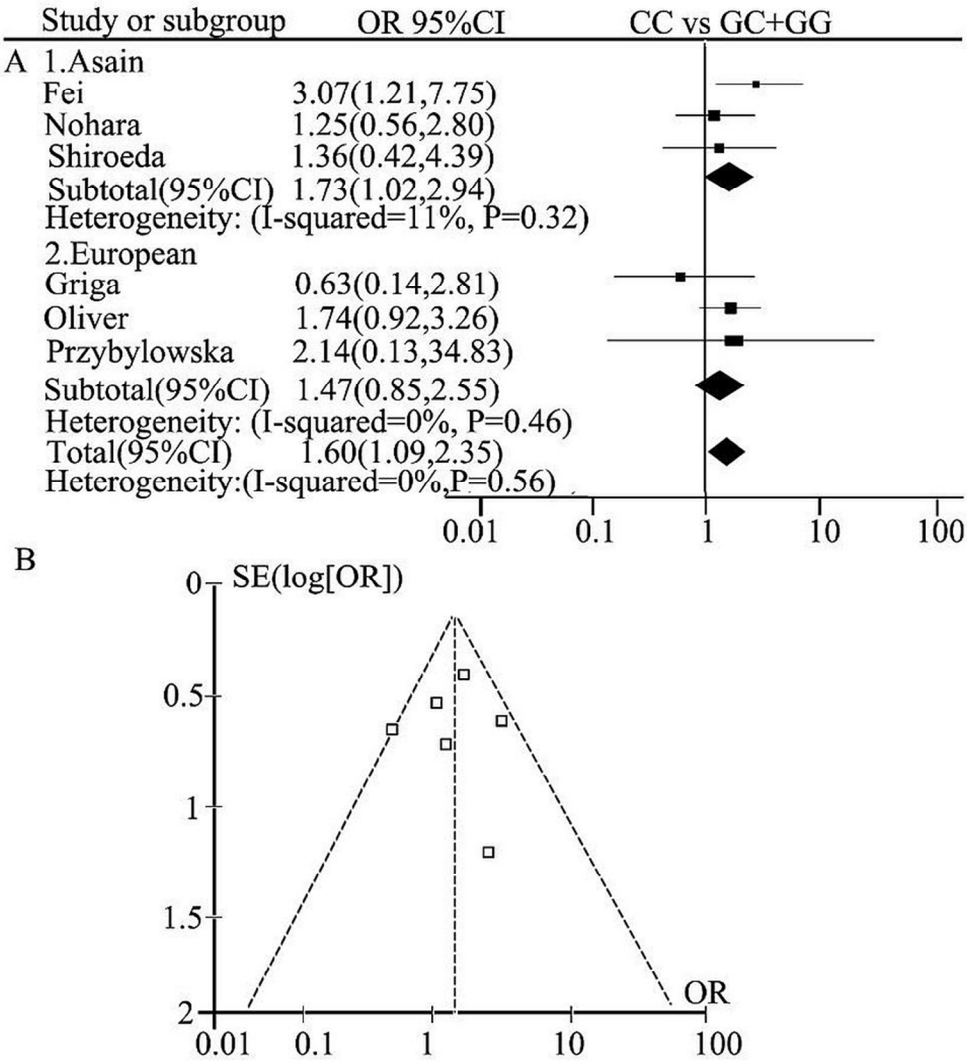

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Macrophage migration inhibitory factor polymorphism and the risk of Ulcerative Colitis and Crohn's Disease in Asian and European populations: A meta-analysis

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Macrophage migration inhibitory factor polymorphism and the risk of Ulcerative Colitis and Crohn's Disease in Asian and European populations: A meta-analysis

Ning-Bo Hao[1], Ya Fei He[1], Gang Luo[1], Xin Yong[1], Yao Zhang[2, 3,*] and Shi-Ming Yang[1, 4, *]

[1] Department of Gastroenterology, Xinqiao Hospital, Third Military Medical University; Chongqing 400038, P.R. China

[2] Department of Epidemiology, Third Military Medical University, Chongqing 400038, P.R. China

[3] The Evidence Based Medicine and Clinic Epidemiology Center, Third Military Medical University, Chongqing, 400038, P.R. China

[4] Chongqing Key Laboratory for Diseases Proteomics, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

Hao and He contributed equally to this study.

[*] To whom requests for reprints should be addressed at the Department of Gastroenterology, Third Military Medical University, Chongqing 400037, China. Phone: 86-023-68754678; E-mail: shimingyang@yahoo.com or the Department of Epidemiology, Third Military Medical University, Chongqing 400038, P. R. China sydzy2003@yahoo.com.cn

Running Title: MIF -173 G/C polymorphism and the risk of IBD

Abbreviations: MIF, macrophage migration inhibitory factor; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; OR, odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

Key Words: Crohn's disease, gene polymorphism, inflammatory bowel disease, macrophage migration inhibitory factor, ulcerative colitis.

Abstract

Objective: To determine whether macrophage migration inhibitory factor (MIF) gene polymorphism is associated with the risk of inflammatory bowel disease (IBD).

Design: System review and meta-analysis.

Methods: MEDLINE, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical Literature database (CBM) were searched for the case-control trails for MIF and IBD. All the studies included into this manuscript met the inclusion and exclusion criteria. An odds ratio (OR) analysis using a 95% confidence interval (CI) was employed to assess the association of the MIF-173 G/C polymorphism with IBD susceptibility.

Results: There was a significant association between the MIF-173 G/C gene polymorphism and IBD in the total population under both the recessive model (CC vs. GC+GG) (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and the codominant model (CC vs. GG) (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). In the stratified analysis by ethnicity, significantly increased risks were observed for Asians using the recessive (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and codominant models (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). Within the subgroups of UC and CD, significant differences were observed regarding UC using the recessive (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and codominant models (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). In the stratified analysis by ethnicity for UC, significant differences were observed regarding CC in Asians vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity).

Conclusion: The meta-analysis suggested that the MIF-173 G/C polymorphism contributed to the susceptibility of IBD. When considering the subgroups of ethnicity and UC and CD, the results suggested that the polymorphism is more significant for UC in Asians.

Article Summary

Article Focus:

1. Examines whether MIF gene polymorphism is associated with IBD, UC or CD.
2. Do Asians and Europeans have different gene mutation points?
3. Are UC and CD caused by the same gene mutation points?

Key messages:

1. It was demonstrated that MIF gene polymorphism is associated with IBD.
2. In Asia, it was found that MIF gene polymorphism is associated with IBD, especially for UC. However, in European, no association was found.
3. In Asia, the main IBD patients were UC, while in European the main patients were CD.

Strengths and limitations of this study:

1. A large number of patients were concluded in this manuscript, which is important when examining the association between MIF and IBD.
2. All the articles included in this manuscript must conform that all controls was in Hardy–Weinberg equilibrium.
3. It still needs to demonstrate that MIF gene polymorphism was associated with IBD in other places such as Africa and South and North America.

Introduction

Inflammatory bowel disease (IBD) currently represents one of the most common health problems worldwide. The highest incidence rates and prevalence of ulcerative colitis (UC) and Crohn's disease (CD) have been reported in northern Europe, the UK, and North America [1]. In addition, in low-incidence areas, such as Asia, southern Europe, and most developing countries, rates also continue to rise [2]. However, the etiology and pathogenesis are still unknown.

Our current understanding of IBD is a complex disease with a number of contributing factors, such as genetic predisposition, environmental factors, intestinal microbial flora, and an aberrant immune response [1]. It has been demonstrated that many IBD patients have a dysregulated intestinal mucosal T-cell-mediated immune response, specifically involving CD4 T helper type-1 (Th1) lymphocytes, which leads to the production of Th1-associated pro-inflammatory cytokines, such as interferon-γ (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-alpha (TNF-α)[3].

In addition, another cytokine, macrophage migration inhibitory factor (MIF), is considered to play a critical role in immunity and inflammation. MIF is secreted by a series of immune cells, such as macrophages, dendritic cells, lymphocytes, neutrophils and pituitary cells[4 5]. Once secreted, MIF regulates a broad range of immune and inflammatory activities, including the induction of inflammatory cytokines, such as TNF-α, IFN-γ, IL-1β, IL-12, IL-6 and, CXCL8 (also known as IL-8), among others [6]. However, if the MIF gene is mutated, it will disturb the immune balance in the microenvironment. It has been demonstrated that MIF gene mutation

is associated with many autoimmune diseases, such as rheumatoid arthritis, glomerulonephritis, and inflammatory bowel diseases [7-11]. MIF genotyping studies have focused on the -794 $CATT_{(5–8)}$ microsatellite and the -173 G/C polymorphism[12]. Donn et al first reported that the MIF 173 polymorphism is a risk factor for juvenile idiopathic arthritis [13]. Consequently, Baugh et al reported the association between the -794 $CATT_{(5–8)}$ microsatellite and disease severity in patients with rheumatoid arthritis[14]. In addition, it has also been reported that the MIF gene polymorphism is a risk factor for other immune diseases, such as atrophy, asthma and sarcoidosis in erythema nodosum patients[12]. Therefore, it is important to explore whether MIF gene polymorphisms are associated with IBD. However, the research results are not in agreement. Thomas and colleagues found that there was no significant difference between IBD patients and controls with the MIF gene type [15]. In other articles, the MIF gene polymorphism has been reported to be a risk factor for IBD [16 17]. Because most of the articles on MIF gene polymorphisms and IBD studied the -173 G/C polymorphism, we performed a meta-analysis to determine whether the MIF -173 G/C polymorphism is a risk factor for IBD. Our analysis reveals that the MIF -173 G/C polymorphism is a risk factor for IBD, especially for UC in Asians.

Materials and Methods

Search Strategy

This meta-analysis followed the proposal of the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group and was performed by searching PubMed, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical

Literature database (CBM) (last search updated in April, 2013) [18]. The search strategy included the following terms: (macrophage migration inhibitory factor [MeSH] or MIF [TEXT WORD] and inflammatory bowel disease [MeSH] or ulcerative colitis [TEXT WORD] or Crohn's disease [TEXT WORD] or IBD [TEXT WORD] or UC [TEXT WORD] or CD [TEXT WORD]). Searches also included scanning reference lists in relevant articles and conference proceedings as well as correspondence with authors when additional data were required. Two reviewers (Hao and He) independently screened the titles and abstracts of each identified reference and categorized papers based on the full text to evaluate their eligibility for inclusion.

Inclusion and Exclusion Criteria

The inclusion criteria for primary studies were as follows: (1) the article evaluated MIF gene polymorphisms and IBD risk; (2) the article included case–control studies or a nested case–control study; (3) the article supplied the number of individual genotypes for the MIF polymorphisms in inflammatory bowel disease cases and controls; and (4) the article demonstrated that the distribution of genotypes among controls was in Hardy–Weinberg equilibrium. In addition, the following exclusion criteria were used. the studies were excluded when 1) it did not provide detailed data such as presented in abstracts, meeting reports and reviews; 2) the studies were repeated or overlapped other publications; 3) the genotype frequency was not reported; and 4) the controls do not meet the assumptions for HWE.

Data Extraction

No paper was included if it did not meet the four inclusion criteria. When the

same study results appeared in several papers, only one study was used in this meta-analysis. Table 1 lists the characteristics of the extracted data, including the first author's name, publication date, region of study, ethnicity of the sample population, number of genotypes, and the total number of cases and controls. The study regions included China, Japan, Spain, Germany and Poland. Data extraction was independently performed by two individuals (Hao and He), and any disagreement was resolved by consensus or by consultation with additional reviewers (Zhang and Yang).

Qualitative Assessment

Quality assessment was performed with the Newcastle-Ottawa quality assessment scale (NOS) for case-control studies. A 'star system' was used to judge data quality based on three broad perspectives: selection, comparability and outcome of interest for case-control studies. Star counts were totaled to compare the study quality in a quantitative fashion[19]. Based on these criteria, the content validity was evaluated by Hao and He, and any disagreement was resolved via discussions between Hao and Luo or with the other authors (Zhang and Yang) for adjudication.

Statistical Analysis

All statistical tests were performed using Revman 5.0 software. Deviations from Hardy–Weinberg equilibrium (HWE) were calculated for the control groups by χ^2 goodness-of-fit. The association between the MIF -173G/C gene polymorphism and IBD was compared by the odds ratio (OR) and the corresponding 95% confidence interval (CI) between the case and control groups. The statistical significance of the

summary OR was determined with the Z test, and P less than 0.05 was considered as statistically significant. The genetic models evaluated for the polymorphism were the dominant model (GC+CC vs. GG), the recessive model (CC vs. GC+GG), the allelic gene model (C vs. G) and the codominant model (CC vs. GG and GC vs. GG).

The heterogeneity between studies was determined by the Chi-square-based Q-test. A P value greater than 0.05 for the Q-test indicated a lack of heterogeneity among the studies. If there was a lack of heterogeneity, the pooled OR estimate of each study was calculated using a fixed-effects model (the Mantel–Haenszel method); otherwise, a random-effects model (the DerSimonian and Laird method) was used [20] [21]. In addition, subgroup analysis stratified by ethnicity and different disease types was also performed. The potential publication bias was estimated by a funnel plot. Egger's linear regression test on the natural logarithm scale of the OR was used to assess the funnel plot asymmetry; the significance was set at the P<0.05 level [22].

Results

Study Characteristics

Figure 1 outlines our search process. First, a total of 123 articles were collected after the initial search with the key words listed previously. After reading the title and abstract, 12 reviews and 1 editorial were excluded. Next, 99 articles were excluded for no association with IBD and the MIF -173 G/C polymorphism. The remaining 11 articles were identified for full-text review, and 4 articles were excluded because of repeated publications. In addition, 1 article was excluded because it deviated from HWE [23]. Finally, 6 articles met our inclusion criteria and were used for the

meta-analysis with 2084 cases and 2288 controls [15-17 24-26]. The basic characteristics of these articles are listed in Table 1. Of the eligible studies, 3/6 articles researched the association of the MIF-173 gene polymorphism with both UC and CD. The remaining 3/6 articles only researched the association of the MIF -173 G/C polymorphism with UC. In total, 3/6 studies were performed in Europeans, and 3/6 were performed in Asians. All studies were cross-sectional case-control studies with sufficient data to calculate the possible relationship between the MIF -173 G/C polymorphism and IBD. All studies were published in English. The distribution of the MIF -173 gene type in IBD, UC and CD is listed in Table 2.

Quantitative Data Synthesis

Table 3 lists the primary results. In the total population, we found a significant difference between the MIF -173 G/C gene polymorphism and the risk of IBD for two variants: CC vs. GC+GG (OR=1.5, CI =1.07-2.14, P=0.02 for heterogeneity) and CC vs. GG (OR=1.54, CI=1.09-2.24, P=0.01 for heterogeneity) (Figures 2 A， B， C and D). No significant difference was observed for the variants of GC+CC vs. GG, GC vs. GG or the allele C vs. G (Table 3).

In the stratified analysis by ethnicity, a significantly increased risk was observed in Asians for CC vs. GC+GG (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and CC vs. GG (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). (Figure 2 A, B) However, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.36, CI=0.86-2.15, P=0.19 for heterogeneity) or CC vs. GG (OR=1.42, CI=0.89-2.24, P=0.14 for heterogeneity).

Because IBD includes UC and CD, we determined whether the role of the MIF -173 G/C gene polymorphism was different between the two diseases. Therefore, we analyzed subgroups of UC and CD. Significant differences were observed in UC for CC vs. GC+GG (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and CC vs. GG (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). (Figure 2 C and D) However, no significant differences were found in CD for CC vs. GC+GG (OR=1.41, CI=0.85-2.36, P=0.19 for heterogeneity) or CC vs. GG (OR=1.44, CI=0.86-2.40, P=0.16 for heterogeneity).

Although IBD includes UC and CD, the cases of IBD in Asians included in our data were mainly UC, and only 15 cases were CD. This may influence the accuracy of the meta-analysis results in the subgroup of ethnicity. Therefore, we analyzed the association of the MIF -173 G/C polymorphism with UC in the subgroup of ethnicity. As shown in Figure 3A, a significant difference was observed in Asians for CC vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity). Similar to previous results, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.47, CI=0.85-2.55, P=0.17 for heterogeneity).

Sensitivity Analyses and Publication Bias

Sensitivity analyses were performed to assess the influence of each individual study on the pooled ORs by the systematic omission of the individual studies from the analyses. the corresponding pooled OR was not materially altered. Begg's funnel plot and the Egger's test were performed to assess the publication bias of the literature. As shown in Figure 3B, the shape of the funnel plot did not reveal obvious asymmetry. The Egger's test was used to provide statistical evidence of funnel plot

symmetry. The results still did not suggest any evidence of publication bias (data not shown).

Discussion

The human MIF gene, which is located on chromosome 22q11.2, is short; it is composed of 3 exons of 205, 173, and 183 bp and 2 introns of 189 and 95 bp [12 27 28]. Four polymorphisms of the human MIF gene have been reported, including a 5–8 CATT tetranucleotide repeat at position -794 $CATT_{(5–8)}$ and 3 single-nucleotide polymorphisms (SNPs) at positions -173 G/C, +254 T/C, and +656 C/G [13 14 29]. However, in IBD, studies on MIF gene polymorphisms have mainly focused on the -173 G/C SNP. Therefore, in this meta-analysis, we mainly discussed the association of the MIF -173 G/C gene polymorphism with the susceptibility to IBD.

In the current meta-analysis of 6 studies consisting of 2084 cases and 2288 controls, we found that the MIF -173 G/C gene polymorphism is significantly associated with IBD susceptibility in both the recessive model (OR: 1.5) and codominant model (OR: 1.54), while no significant associations were found in the dominant model or the allelic model. The results indicated that the MIF -173 G/C polymorphism was a conspicuous high-risk factor for developing IBD in the overall study population.

The second finding of this meta-analysis is that in the subgroup of ethnicity, the MIF -173 G/C gene polymorphism was significantly different in Asians in the recessive (OR: 1.75) and codominant (OR: 1.74) models, while no significant differences were found in Europeans. This finding is consistent with previous results that a gene

polymorphism does not have the same effect in different ethnicities. For example, the TNF-α308A gene polymorphism plays an important role in Asian populations, while no conclusive data on this association exist in European patients [30]. In addition, the best studied genetic variant, a nucleotide oligomerization domain (NOD)2 polymorphism, is present in up to 20% of patients with CD in White and Jewish populations, but major disease-associated variants have not been detected in individuals of Asian descent with CD [31 32].

The third finding of this meta-analysis is that in the subgroup of the two diseases, UC and CD, significant differences were found in the recessive (OR: 1.60) and codominant (OR: 1.64) model for UC, while no difference was found in the recessive or codominant model for CD. This result suggests that the MIF -173 G/C polymorphism seems to be a risk factor for UC but not for CD. However, in another study, Zhang and colleagues confirmed that the peroxisome proliferator-activated receptor gamma (PPARγ) AlaAla genotype is a protective factor against the development of CD, especially in the European Caucasian population, while no significant association was found in the development of UC [33]. Both of these results indicated that the gene polymorphism has different effects in different diseases.

To further determine whether the MIF -173 gene polymorphism for UC was different in different ethnicities, we performed a meta-analysis of UC in the subgroup of ethnicity. A significant difference was found in the recessive model (OR: 1.73) in Asians, while no significant difference was found in Europeans. Although only three studies were included in the sub-group analysis, they all have high quality. So the

results in the sub-group analysis were credible.

In addition, two similar studies had published. However, compared to the two studies [34 35], our study has the special strength. First it has been claimed that the studies included in the meta-analysis should not deviated from HWE. So in our study we didn't include the study from India.[23] Second, in our study, we analyses MIF polymorphism and the risk of UC in Asian and European. Since the results of MIF polymorphism and the risk of IBD may be influenced by the bias because the number of CD in Asian was low. However, the present meta-analysis had several limitations that must be taken into consideration. First, the number of available studies that could be included in this meta-analysis was moderate. Therefore, the results could be influenced by factors such as random error. Therefore, next we will focus on research on the association between gene polymorphisms and IBD in our hospitals. Second, the overall outcomes were based on individual unadjusted ORs, while a more precise evaluation should be adjusted by other potentially suspected factors, including age, sex, and environmental factors.

In summary, the current meta-analysis suggested that the MIF -173 G/C polymorphism contributed to the susceptibility of IBD. In the subgroups of ethnicity and UC and CD, the result suggested that this polymorphism was more significant for UC in Asians.

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Author contributorship statement

1. Hao,and He contributed to conception and design, acquisition of data, or analysis and interpretation of data.
2. Hao and Yong acquisition of data
3. Hao, He, Luo and Zhang analyses and interpreted the data.
4. Hao and Yang drafted the article.
5. All the authors listed in this manuscript approval to publish in ***BMJ OPEN***.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data Sharing Statement

No additional data

Reference

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Legends to Figures

Figure 1 Flow of study identification, inclusion, and exclusion.

Figure 2 Forest plot of IBD risk associated with MIF -173G/C polymorphism for different genetic model. (A) CC vs. GG+GC and (B) CC vs. GG for ethnicity; (C) CC vs. GC+GG and (D) CC vs. GG for UC and CD.

Figure 3 (A) Forest plot of UC risk associated with MIF -173G/C polymorphism for CC vs. GG+GC in Asian and European; (B) Begg's funnel plot of the MIF 173 gene polymorphism and inflammatory bowel disease risk for combined CC vs. GC+GG

Macrophage migration inhibitory factor polymorphism and the risk of Ulcerative Colitis and Crohn's Disease in Asian and European: A meta-analysis of 4372 Subjects

Ning-Bo Hao[1], Ya Fei He[1], Gang Luo[1], Xin Yong[1], Yao Zhang[2, 3,*] and Shi-Ming Yang[1, 4, *]

[1] Department of Gastroenterology, Xinqiao Hospital, Third Military Medical University; Chongqing 400038, P.R. China

[2] Department of Epidemiology, Third Military Medical University, Chongqing 400038, P.R. China

[3] The Evidence Based Medicine and Clinic Epidemiology Center, Third Military Medical University, Chongqing, 400038, P.R. China

[4] Chongqing Key Laboratory for Diseases Proteomics, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

Hao and He contributed equally to this study.

[*] To whom requests for reprints should be addressed at the Department of Gastroenterology, Third Military Medical University, Chongqing 400037, China. Phone: 86-023-68754678; E-mail: shimingyang@yahoo.com or the Department of Epidemiology, Third Military Medical University, Chongqing 400038, P. R. China sydzy2003@yahoo.com.cn

Running Title: MIF -173 G/C polymorphism and the risk of IBD

Abbreviations: MIF, macrophage migration inhibitory factor; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; OR, odds ratio; CI,

confidence interval; HWE, Hardy–Weinberg equilibrium.

Abstract

Objective: To determine whether macrophage migration inhibitory factor (MIF) gene polymorphism is associated with the risk of inflammatory bowel disease (IBD).

Design: System review and meta-analysis.

Methods: MEDLINE, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical Literature database (CBM) were searched for the case-control trails for MIF and IBD. All the studies included into this manuscript met the inclusion and exclusion criteria. An odds ratio (OR) analysis using a 95% confidence interval (CI) was employed to assess the association of the MIF-173 G/C polymorphism with IBD susceptibility.

Results: There was a significant association between the MIF-173 G/C gene polymorphism and IBD in the total population under both the recessive model (CC vs. GC+GG) (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and the codominant model (CC vs. GG) (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). In the stratified analysis by ethnicity, significantly increased risks were observed for Asians using the recessive (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and codominant models (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). Within the subgroups of UC and CD, significant differences were observed regarding UC using the recessive (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and codominant models (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). In the stratified analysis by ethnicity for UC, significant differences were observed regarding CC in Asians vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity).

Conclusion: The meta-analysis suggested that the MIF-173 G/C polymorphism contributed to the susceptibility of IBD. When considering the subgroups of ethnicity and UC and CD, the results suggested that the polymorphism is more significant for UC in Asians.

Key Words: Crohn's disease, gene polymorphism, inflammatory bowel disease, macrophage migration inhibitory factor, ulcerative colitis.

Introduction

Inflammatory bowel disease (IBD) currently represents one of the most common health problems worldwide. The highest incidence rates and prevalence of ulcerative colitis (UC) and Crohn's disease (CD) have been reported in northern Europe, the UK, and North America [1]. In addition, in low-incidence areas, such as Asia, southern Europe, and most developing countries, rates also continue to rise [2]. However, the etiology and pathogenesis are still unknown.

Our current understanding of IBD is a complex disease with a number of contributing factors, such as genetic predisposition, environmental factors, intestinal microbial flora, and an aberrant immune response [1]. It has been demonstrated that many IBD patients have a dysregulated intestinal mucosal T-cell-mediated immune response, specifically involving CD4 T helper type-1 (Th1) lymphocytes, which leads to the production of Th1-associated pro-inflammatory cytokines, such as interferon-γ (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-alpha (TNF-α)[3].

In addition, another cytokine, macrophage migration inhibitory factor (MIF), is considered to play a critical role in immunity and inflammation. MIF is secreted by a series of immune cells, such as macrophages, dendritic cells, lymphocytes, neutrophils and pituitary cells[4 5]. Once secreted, MIF regulates a broad range of immune and inflammatory activities, including the induction of inflammatory cytokines, such as TNF-α, IFN-γ, IL-1β, IL-12, IL-6 and, CXCL8 (also known as

IL-8), among others [6]. However, if the MIF gene is mutated, it will disturb the immune balance in the microenvironment. It has been demonstrated that MIF gene mutation is associated with many autoimmune diseases, such as rheumatoid arthritis, glomerulonephritis, and inflammatory bowel diseases [7-11]. MIF genotyping studies have focused on the -794 $CATT_{(5–8)}$ microsatellite and the -173 G/C polymorphism[12]. Donn et al first reported that the MIF 173 polymorphism is a risk factor for juvenile idiopathic arthritis [13]. Consequently, Baugh et al reported the association between the -794 $CATT_{(5–8)}$ microsatellite and disease severity in patients with rheumatoid arthritis[14]. In addition, it has also been reported that the MIF gene polymorphism is a risk factor for other immune diseases, such as atrophy, asthma and sarcoidosis in erythema nodosum patients[12]. Therefore, it is important to explore whether MIF gene polymorphisms are associated with IBD. However, the research results are not in agreement. Thomas and colleagues found that there was no significant difference between IBD patients and controls with the MIF gene type [15]. In other articles, the MIF gene polymorphism has been reported to be a risk factor for IBD [16 17]. Because most of the articles on MIF gene polymorphisms and IBD studied the -173 G/C polymorphism, we performed a meta-analysis to determine whether the MIF -173 G/C polymorphism is a risk factor for IBD. Our analysis reveals that the MIF -173 G/C polymorphism is a risk factor for IBD, especially for UC in Asians.

Materials and Methods

Search Strategy

This meta-analysis followed the proposal of the Meta-analysis Of Observational

Studies in Epidemiology (MOOSE) group and was performed by searching PubMed, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical Literature database (CBM) (last search updated in April, 2013) [18]. The search strategy included the following terms: (macrophage migration inhibitory factor [MeSH] or MIF [TEXT WORD] and inflammatory bowel disease [MeSH] or ulcerative colitis [TEXT WORD] or Crohn's disease [TEXT WORD] or IBD [TEXT WORD] or UC [TEXT WORD] or CD [TEXT WORD]). Searches also included scanning reference lists in relevant articles and conference proceedings as well as correspondence with authors when additional data were required. Two reviewers (Hao and He) independently screened the titles and abstracts of each identified reference and categorized papers based on the full text to evaluate their eligibility for inclusion.

Inclusion and Exclusion Criteria

The inclusion criteria for primary studies were as follows: (1) the article evaluated MIF gene polymorphisms and IBD risk; (2) the article included case–control studies or a nested case–control study; (3) the article supplied the number of individual genotypes for the MIF polymorphisms in inflammatory bowel disease cases and controls; and (4) the article demonstrated that the distribution of genotypes among controls was in Hardy–Weinberg equilibrium. In addition, the following exclusion criteria were used. the studies were excluded when 1) it did not provide detailed data such as presented in abstracts, meeting reports and reviews; 2) the studies were repeated or overlapped other publications; 3) the genotype frequency was not reported; and 4) the controls do not meet the assumptions for HWE.

Data Extraction

No paper was included if it did not meet the four inclusion criteria. When the same study results appeared in several papers, only one study was used in this meta-analysis. Table 1 lists the characteristics of the extracted data, including the first author's name, publication date, region of study, ethnicity of the sample population, number of genotypes, and the total number of cases and controls. The study regions included China, Japan, Spain, Germany and Poland. Data extraction was independently performed by two individuals (Hao and He), and any disagreement was resolved by consensus or by consultation with additional reviewers (Zhang and Yang).

Qualitative Assessment

Quality assessment was performed with the Newcastle-Ottawa quality assessment scale (NOS) for case-control studies. A 'star system' was used to judge data quality based on three broad perspectives: selection, comparability and outcome of interest for case-control studies. Star counts were totaled to compare the study quality in a quantitative fashion[19]. Based on these criteria, the content validity was evaluated by Hao and He, and any disagreement was resolved via discussions between Hao and Luo or with the other authors (Zhang and Yang) for adjudication.

Statistical Analysis

All statistical tests were performed using Revman 5.0 software. Deviations from Hardy–Weinberg equilibrium (HWE) were calculated for the control groups by χ^2 goodness-of-fit. The association between the MIF -173G/C gene polymorphism and IBD was compared by the odds ratio (OR) and the corresponding 95% confidence

interval (CI) between the case and control groups. The statistical significance of the summary OR was determined with the Z test, and P less than 0.05 was considered as statistically significant. The genetic models evaluated for the polymorphism were the dominant model (GC+CC vs. GG), the recessive model (CC vs. GC+GG), the allelic gene model (C vs. G) and the codominant model (CC vs. GG and GC vs. GG).

The heterogeneity between studies was determined by the Chi-square-based Q-test. A P value greater than 0.05 for the Q-test indicated a lack of heterogeneity among the studies. If there was a lack of heterogeneity, the pooled OR estimate of each study was calculated using a fixed-effects model (the Mantel–Haenszel method); otherwise, a random-effects model (the DerSimonian and Laird method) was used [20 21]. In addition, subgroup analysis stratified by ethnicity and different disease types was also performed. The potential publication bias was estimated by a funnel plot. Egger's linear regression test on the natural logarithm scale of the OR was used to assess the funnel plot asymmetry; the significance was set at the P<0.05 level [22].

Results

Study Characteristics

Figure 1 outlines our search process. First, a total of 123 articles were collected after the initial search with the key words listed previously. After reading the title and abstract, 12 reviews and 1 editorial were excluded. Next, 99 articles were excluded for no association with IBD and the MIF -173 G/C polymorphism. The remaining 11 articles were identified for full-text review, and 4 articles were excluded because of repeated publications. In addition, 1 article was excluded because it deviated from

HWE [23]. Finally, 6 articles met our inclusion criteria and were used for the meta-analysis with 2084 cases and 2288 controls [15-17 24-26]. The basic characteristics of these articles are listed in Table 1. Of the eligible studies, 3/6 articles researched the association of the MIF-173 gene polymorphism with both UC and CD. The remaining 3/6 articles only researched the association of the MIF -173 G/C polymorphism with UC. In total, 3/6 studies were performed in Europeans, and 3/6 were performed in Asians. All studies were cross-sectional case-control studies with sufficient data to calculate the possible relationship between the MIF -173 G/C polymorphism and IBD. All studies were published in English. The distribution of the MIF -173 gene type in IBD, UC and CD is listed in Table 2.

Quantitative Data Synthesis

Table 3 lists the primary results. In the total population, we found a significant difference between the MIF -173 G/C gene polymorphism and the risk of IBD for two variants: CC vs. GC+GG (OR=1.5, CI =1.07-2.14, P=0.02 for heterogeneity) and CC vs. GG (OR=1.54, CI=1.09-2.24, P=0.01 for heterogeneity) (Figures 2 A， B， C and D). No significant difference was observed for the variants of GC+CC vs. GG, GC vs. GG or the allele C vs. G (Table 3).

In the stratified analysis by ethnicity, a significantly increased risk was observed in Asians for CC vs. GC+GG (OR=1.75, CI=1.04-2.95, P=0.04 for heterogeneity) and CC vs. GG (OR=1.74, CI=1.02-2.97, P=0.04 for heterogeneity). (Figure 2 A, B) However, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.36, CI=0.86-2.15, P=0.19 for heterogeneity) or CC vs. GG (OR=1.42,

CI=0.89-2.24, P=0.14 for heterogeneity).

Because IBD includes UC and CD, we determined whether the role of the MIF -173 G/C gene polymorphism was different between the two diseases. Therefore, we analyzed subgroups of UC and CD. Significant differences were observed in UC for CC vs. GC+GG (OR=1.60, CI=1.09-2.35, P=0.02 for heterogeneity) and CC vs. GG (OR=1.64, CI=1.12-2.41, P=0.01 for heterogeneity). (Figure 2 C and D) However, no significant differences were found in CD for CC vs. GC+GG (OR=1.41, CI=0.85-2.36, P=0.19 for heterogeneity) or CC vs. GG (OR=1.44, CI=0.86-2.40, P=0.16 for heterogeneity).

Although IBD includes UC and CD, the cases of IBD in Asians included in our data were mainly UC, and only 15 cases were CD. This may influence the accuracy of the meta-analysis results in the subgroup of ethnicity. Therefore, we analyzed the association of the MIF -173 G/C polymorphism with UC in the subgroup of ethnicity. As shown in Figure 3A, a significant difference was observed in Asians for CC vs. GC+GG (OR=1.73, CI=1.02-2.94, P=0.04 for heterogeneity). Similar to previous results, no significant difference was observed in Europeans for CC vs. GC+GG (OR=1.47, CI=0.85-2.55, P=0.17 for heterogeneity).

Sensitivity Analyses and Publication Bias

Sensitivity analyses were performed to assess the influence of each individual study on the pooled ORs by the systematic omission of the individual studies from the analyses. the corresponding pooled OR was not materially altered. Begg's funnel plot and the Egger's test were performed to assess the publication bias of the literature. As

shown in Figure 3B, the shape of the funnel plot did not reveal obvious asymmetry. The Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias (data not shown).

Discussion

The human MIF gene, which is located on chromosome 22q11.2, is short; it is composed of 3 exons of 205, 173, and 183 bp and 2 introns of 189 and 95 bp [12 27 28]. Four polymorphisms of the human MIF gene have been reported, including a 5–8 CATT tetranucleotide repeat at position -794 $CATT_{(5–8)}$ and 3 single-nucleotide polymorphisms (SNPs) at positions -173 G/C, +254 T/C, and +656 C/G [13 14 29]. However, in IBD, studies on MIF gene polymorphisms have mainly focused on the -173 G/C SNP. Therefore, in this meta-analysis, we mainly discussed the association of the MIF -173 G/C gene polymorphism with the susceptibility to IBD.

In the current meta-analysis of 6 studies consisting of 2084 cases and 2288 controls, we found that the MIF -173 G/C gene polymorphism is significantly associated with IBD susceptibility in both the recessive model (OR: 1.5) and codominant model (OR: 1.54), while no significant associations were found in the dominant model or the allelic model. The results indicated that the MIF -173 G/C polymorphism was a conspicuous high-risk factor for developing IBD in the overall study population.

The second finding of this meta-analysis is that in the subgroup of ethnicity, the MIF -173 G/C gene polymorphism was significantly different in Asians in the recessive (OR: 1.75) and codominant (OR: 1.74) models, while no significant

differences were found in Europeans. This finding is consistent with previous results that a gene polymorphism does not have the same effect in different ethnicities. For example, the TNF-α308A gene polymorphism plays an important role in Asian populations, while no conclusive data on this association exist in European patients [30]. In addition, the best studied genetic variant, a nucleotide oligomerization domain (NOD)2 polymorphism, is present in up to 20% of patients with CD in White and Jewish populations, but major disease-associated variants have not been detected in individuals of Asian descent with CD [31 32].

The third finding of this meta-analysis is that in the subgroup of the two diseases, UC and CD, significant differences were found in the recessive (OR: 1.60) and codominant (OR: 1.64) model for UC, while no difference was found in the recessive or codominant model for CD. This result suggests that the MIF -173 G/C polymorphism seems to be a risk factor for UC but not for CD. However, in another study, Zhang and colleagues confirmed that the peroxisome proliferator-activated receptor gamma (PPARγ) AlaAla genotype is a protective factor against the development of CD, especially in the European Caucasian population, while no significant association was found in the development of UC [33]. Both of these results indicated that the gene polymorphism has different effects in different diseases.

To further determine whether the MIF -173 gene polymorphism for UC was different in different ethnicities, we performed a meta-analysis of UC in the subgroup of ethnicity. A significant difference was found in the recessive model (OR: 1.73) in Asians, while no significant difference was found in Europeans. Although only three

studies were included in the sub-group analysis, they all have high quality. So the results in the sub-group analysis were credible.

In addition, two similar studies had published. However, compared to the two studies [34 35], our study has the special strength. First it has been claimed that the studies included in the meta-analysis should not deviated from HWE. So in our study we didn't include the study from India.[23] Second, in our study, we analyses MIF polymorphism and the risk of UC in Asian and European. Since the results of MIF polymorphism and the risk of IBD may be influenced by the bias because the number of CD in Asian was low. However, the present meta-analysis had several limitations that must be taken into consideration. First, the number of available studies that could be included in this meta-analysis was moderate. Therefore, the results could be influenced by factors such as random error. Therefore, next we will focus on research on the association between gene polymorphisms and IBD in our hospitals. Second, the overall outcomes were based on individual unadjusted ORs, while a more precise evaluation should be adjusted by other potentially suspected factors, including age, sex, and environmental factors.

In summary, the current meta-analysis suggested that the MIF -173 G/C polymorphism contributed to the susceptibility of IBD. In the subgroups of ethnicity and UC and CD, the result suggested that this polymorphism was more significant for UC in Asians.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Legends to Figures

Figure 1 Flow of study identification, inclusion, and exclusion.

Figure 2 Forest plot of IBD risk associated with MIF -173G/C polymorphism for different genetic model. (A) CC vs. GG+GC and (B) CC vs. GG for ethnicity; (C) CC vs. GC+GG and (D) CC vs. GG for UC and CD.

Figure 3 (A) Forest plot of UC risk associated with MIF -173G/C polymorphism for CC vs. GG+GC in Asian and European; (B) Begg's funnel plot of the MIF 173 gene polymorphism and inflammatory bowel disease risk for combined CC vs. GC+GG

Article Summary

Article Focus:

1. Examines whether MIF gene polymorphism is associated with IBD, UC or CD.
2. Do Asians and Europeans have different gene mutation points?
3. Are UC and CD caused by the same gene mutation points?

Key messages:

1. It was demonstrated that MIF gene polymorphism is associated with IBD.
2. In Asia, it was found that MIF gene polymorphism is associated with IBD, especially for UC. However, in European, no association was found.
3. In Asia, the main IBD patients were UC, while in European the main patients

were CD.

Strengths and limitations of this study:

1. A large number of patients were concluded in this manuscript, which is important when examining the association between MIF and IBD.
2. All the articles included in this manuscript must conform that all controls was in Hardy–Weinberg equilibrium.
3. It still needs to demonstrate that MIF gene polymorphism was associated with IBD in other places such as Africa and South and North America.

Author contributorship statement

1. Hao,and He contributed to conception and design, acquisition of data, or analysis and interpretation of data.
2. Hao and Yong acquisition of data
3. Hao, He, Luo and Zhang analyses and interpreted the data.
4. Hao and Yang drafted the article.
5. All the authors listed in this manuscript approval to publish in ***BMJ OPEN***.

Table1 Characteristics of studies included in meta-analysis

First author	Years	Country	Ethnicity	Case number(n)	Control Number (n)	Newcastle-Ottawa Score	Genotyping
Griaga	2007	Germany	European	259	489	7/9	PCR-RFLP
Shiroeda	2010	Japan	Asian	111	209	6/9	PCR-SSCP
Fei	2008	China	Asian	99	142	9/9	PCR-RFLP tetra-primer ARMS
Oliver	2007	Spain	European	1295	887	8/9	PCR-RFLP
Przybyłowska	2011	Poland	European	99	123	6/9	PCR-RFLP
Nohara	2004	Japan	Asian	221	438	7/9	PCR-RFLP PCR-SSCP

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism， ARMS： amplification refractory mutation system

PCR-SSCP, polymerase chain reaction-single-strand conformation polymorphism，

Table 2: Genotypes and allele frequencies of MIF-173G/C genes in patients and controls

Author	Year	Case					Control					Sample size	HWE（P）
		GG	GC	CC	G	C	GG	GC	CC	G	C		
IBD													
Fei	2008	52	32	15	136	62	79	55	8	213	71	99/142	0.70
Griaga	2007	188	67	4	443	75	318	156	15	792	186	259/489	0.43
Nohara	2004	135	76	10	346	96	288	134	16	710	166	221/438	0.93
Shiroeda	2010	69	37	5	175	47	126	76	7	328	90	111/209	0.27
Przybyłowska	2011	66	31	2	163	35	99	23	1	221	25	99/123	0.79
Oliver	2007	907	343	45	2157	433	681	188	18	1550	224	1295/887	0.24
UC													
Fei	2008	44	27	13	115	53	79	55	8	213	71	84/142	0.70
Griaga	2007	72	28	2	172	32	318	156	15	792	186	102/489	0.43
Nohara	2004	135	76	10	346	96	288	134	16	710	166	221/438	0.93
Oliver	2007	441	171	22	1053	215	681	188	18	1550	224	634/887	0.24

Przybyłowska	2011	38	19	1	95	21	99	23	1	221	25	58/123	0.79
Shiroeda	2010	69	37	5	175	47	126	76	7	328	90	111/209	0.27
CD													
Fei	2008	8	5	2	21	9	79	55	8	213	71	15/142	0.70
Griaga	2007	116	39	2	271	43	318	156	15	792	186	157/489	0.43
Oliver	2007	466	172	23	1104	218	681	188	18	1550	224	661/887	0.24
Przybyłowska	2011	28	12	1	68	14	99	23	1	221	25	41/123	0.79

HWE: Hardy-Weinberg Equilibrium

Table 3 Summary of different comparative results

	Study	Sample size		No. of	Test of association			Model	Heterogeneity		
		case	control	studies	OR (95% CI)	*Z*	*P* Value		χ^2	*P* Value	I^2 (%)
GC+CC vs. GG	Overall	2084	2288	6	1.15（0.87-1.52）	0.97	0.33	R	17.04	0.004	71.0
	Asian	431	789	3	1.12 (0.88 – 1.44)	0.90	0.37	F	0.90	0.64	0.0
	European	1653	1499	3	1.23 (0.70 – 2.14)	0.71	0.48	R	15.75	0.0004	87.0
	UC	1210	2288	6	1.24 (1.06 – 1.44)	2.76	0.006	F	9.69	0.08	48.0
	CD	874	1641	4	1.13 (0.69 – 1.87)	0.49	0.62	F	11.46	0.009	74.0
CC vs. GC+GG	Overall	2084	2282	6	1.51(1.07-2.14)	2.34	0.02	F	6.72	0.24	26.0
	Asian	431	789	3	1.75(1.04 – 2.95)	2.10	0.04	F	2.21	0.33	9.0
	European	1653	1499	3	1.36 (0.86 – 2.15)	1.31	0.19	F	4.16	0.12	52.0
	UC	1210	2288	6	1.60 (1.09 – 2.35)	2.42	0.02	F	3.92	0.56	0.0
	CD	874	1641	4	1.41 (0.85 – 2.36)	1.32	0.19	F	3.91	0.27	23.0
CC vs. GG	Overall	1498	1656	6	1.54（1.09-2.24）	2.43	0.01	F	7.30	0.20	32.0
	Asian	286	524	3	1.74（1.02– 2.97）	2.04	0.04	F	1.72	0.42	0.0
	European	1212	1132	3	1.42 (0.89 – 2.24)	1.49	0.14	F	5.38	0.07	63.0
	UC	852	1656	6	1.64 (1.12 – 2.41)	2.52	0.01	F	3.88	0.57	0.0
	CD	646	1219	4	1.44 (0.86 – 2.40)	1.40	0.16	F	4.68	0.20	36.0
GC vs. GG	Overall	2003	1972	6	1.40（1.00-1.95)	1.97	0.05	R	18.46	0.002	73.0
	Asian	401	810	3	1.02 (0.79 – 1.31)	0.14	0.89	F	1.01	0.60	0.0
	European	1602	1465	3	1.21 (0.72 – 2.03)	0.73	0.47	R	12.77	0.002	84.0
	UC	1157	2223	6	1.20 (1.02 – 1.40)	2.20	0.03	F	9.64	0.09	48.0
	CD	846	1599	4	1.09 (0.69 – 1.74)	0.39	0.70	R	9.13	0.03	67.0
C vs. G	Overall	4168	4576	6	1.17 (0.91 – 1.50)	1.25	0.21	R	18.47	0.002	73.0
	Asian	862	1578	3	1.17 (0.96 – 1.43)	1.54	0.12	F	1.36	0.51	0.0
	European	3306	2998	3	1.20 (0.72 – 2.01)	0.70	0.49	R	17.04	0.0002	88.0
	UC	2420	4576	6	1.24 (1.09 – 1.41)	3.20	0.001	F	9.51	0.08	50.0
	CD	1748	3282	4	1.16(0.73 – 1.83)	0.63	0.53	R	12.78	0.005	77.0

* vs.: versus; R: random-effects model; F: fixed-effects model.

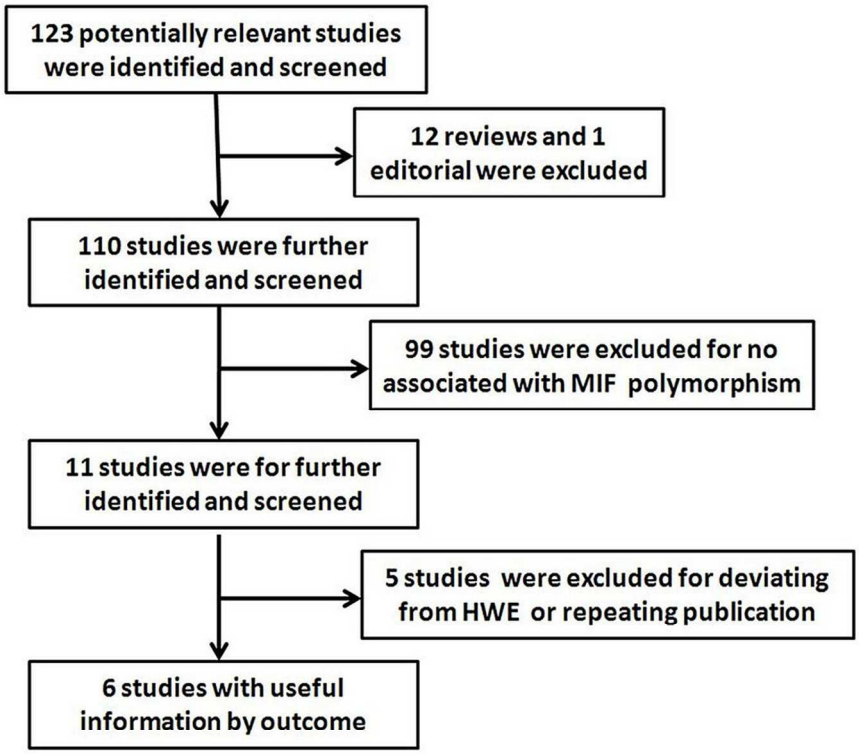

Figure 1

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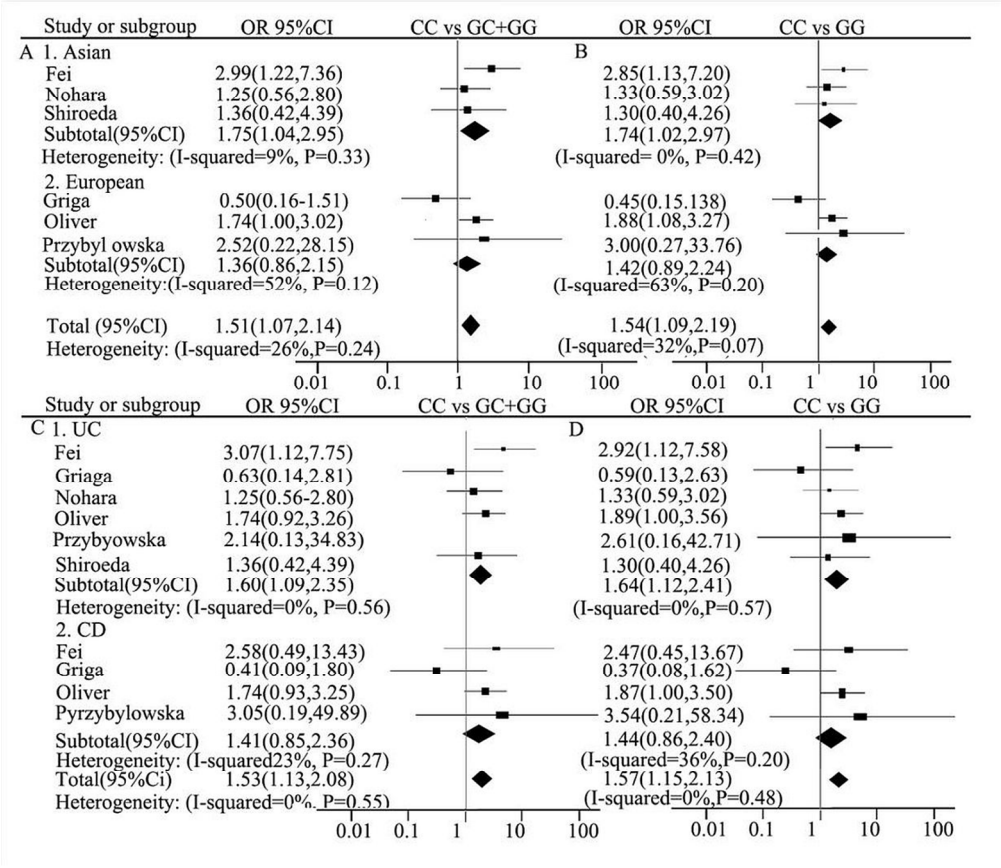

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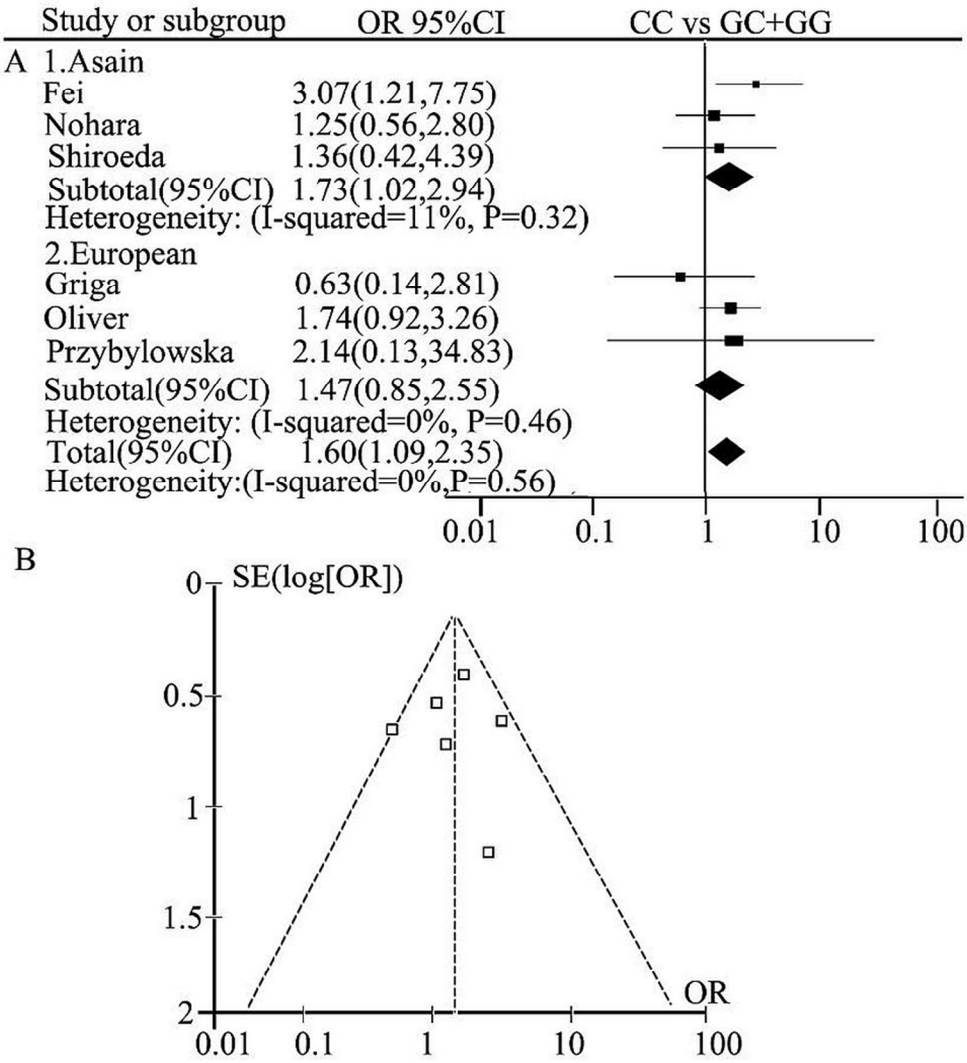

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