GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Correlation between *IRGM* genetic polymorphisms and Crohn's disease risk: a meta-analysis of case-control studies

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Genet. Mol. Res. 13 (4): 10741-10753 (2014)
Received November 8, 2013
Accepted April 28, 2014
Published December 18, 2014
DOI http://dx.doi.org/10.4238/2014.December.18.15

ABSTRACT. This meta-analysis was performed to evaluate the relationships between single-nucleotide polymorphisms (SNPs) in the immunity-related GTPase M (*IRGM*) gene and the risk of Crohn's disease (CD). Eleven case-control studies were included, for a total of 5183 CD patients and 5571 healthy controls. Three common SNPs (rs13361189 C>T, rs10065172 C>T, and rs4958847 A>G) in the *IRGM* gene were assessed. We found that the *IRGM* rs13361189 polymorphism was significantly associated with an increased risk of CD [C allele *vs* T allele: odds ratio (OR) = 1.30, 95% confidence interval (CI) = 1.05-1.61, P = 0.017; CC + CT *vs* TT: OR = 1.32, 95%CI = 1.06-1.64, P = 0.013]. However, we observed no correlation between the rs10065172 and rs4958847 polymorphisms in the *IRGM* gene with susceptibility to CD (all P > 0.05). Subgroup analysis by ethnicity revealed significant associations between *IRGM* genetic polymorphisms and an increased risk of CD

among Caucasian populations (C allele *vs* T allele: OR = 1.22, 95%CI = 1.07-1.40, P = 0.004; CC + CT *vs* TT: OR = 1.22, 95%CI = 1.05-1.41, P = 0.009), but not among Asian populations (all P > 0.05). Meta-regression analysis also confirmed that ethnic differences may be an important source of heterogeneity (P = 0.003). Our meta-analysis results indicate that the *IRGM* rs13361189 polymorphism contributes to the susceptibility to CD. Thus, the *IRGM* rs13361189 polymorphism is promising as a biomarker for early diagnosis of CD. However, the *IRGM* rs10065172 and rs4958847 polymorphisms may not be the major determinants of CD risk.

Key words: Crohn's disease; *IRGM*; Single nucleotide polymorphism; Meta-analysis

INTRODUCTION

Crohn's disease (CD), an inflammatory bowel disease, is a chronic progressive destructive illness that primarily affects any part of the gastrointestinal tract from the mouth to the anus and causes a wide variety of symptoms (Beaugerie et al., 2009; Smith et al., 2009). Furthermore, the incidence and prevalence of CD have increased in recent years in both adults and children (Latiano et al., 2010). Although the causes and etiology of CD are currently unknown, both genetic and environmental factors as well as immune interactions contribute to the development and progression of CD (Todd, 2010). It is well known that genetic variations in immunity-related genes influence individual susceptibility to CD (Parkes et al., 2007).

Immunity-related GTPase family M protein (IRGM), also known as interferon-inducible protein 1 (IFI1), belongs to the p47 immunity-related GTPase family and plays a crucial role in innate resistance to intracellular pathogens (Moon et al., 2013). Importantly, IRGM proteins function to regulate autophagic flux, which influences the localization of glycophorin-binding proteins and potentially other factors that direct cell-autonomous immune resistance (Traver et al., 2011). The human *IRGM* gene, located on chromosome 5q33.1, contains 5 exons and 4 introns (Latiano et al., 2009). Genetic mutations in *IRGM* may impact autophagic clearance of intracellular bacteria, lead to intracellular bacterial survival, activate the immune response, and thus increase susceptibility to CD (Henry et al., 2010; Brest et al., 2011). Therefore, we hypothesized that single-nucleotide polymorphisms (SNPs) in the *IRGM* gene are functional and are associated with the development of CD (Jung et al., 2012). Among these SNPs in the *IRGM* gene, rs13361189 (C>T), rs10065172 (C>T), and rs4958847 (A>G) are the most common (Latiano et al., 2009; Brest et al., 2011; Gardet and Xavier, 2012). Large quantities of evidence have indicated that *IRGM* genetic polymorphisms increase the risk of CD (Roberts et al., 2008; Wolfkamp et al., 2010), but the results of these studies have been contradictory (Meggyesi et al., 2010; Zheng and Pang, 2012). Therefore, we performed this meta-analysis to evaluate the relationships between *IRGM* genetic polymorphisms and CD risk.

MATERIAL AND METHODS

Literature search

The PubMed, CISCOM, CINAHL, Web of Science, Google Scholar, EBSCO,

Cochrane Library, and CBM databases were searched from their inception through October 1, 2013 without language restrictions. The initial literature search used the following key words and MeSH terms: ["SNP" or "mutation" or "genetic polymorphism" or "variation" or "polymorphism" or "single-nucleotide polymorphism" or "variant"] and ["Crohn's disease" or "CD"] and ["human immunity-related GTPase M" or "*IRGM*"]. A manual search of the references of included studies was conducted to identify other potentially eligible studies.

Selection criteria

Studies included in our meta-analysis met the following criteria: 1) the study design was a clinical cohort or case-control study; 2) the study examined the relationships between *IRGM* genetic polymorphisms and susceptibility to CD; 3) all patients conformed to the diagnostic criteria of CD; and 4) the study provided sufficient information regarding the frequencies of *IRGM* genetic polymorphisms. Studies that did not meet all of the inclusion criteria were excluded from analysis. The most recent or the largest sample size publication was included when the authors published several studies using data from the same subjects.

Data extraction

Using a standardized form, relevant data were systematically extracted from all included studies by 2 researchers. The standardized form included the following items: language of publication, publication year of article, the first author's surname, geographical location, design of study, sample size, the source of the subjects, allele frequencies, source of samples, genotyping method of SNP, and evidence of Hardy-Weinberg equilibrium (HWE) in healthy controls.

Quality assessment

We evaluated the methodological quality of the included studies according to Newcastle-Ottawa Scale (NOS) criteria (Stang, 2010). The NOS criteria included 3 aspects: 1) subject selection: 0-4 scores; 2) comparability of subject: 0-2 scores; and 3) clinical outcome: 0-3 scores. NOS scores ranged from 0-9; a score $\geq$7 indicated good quality.

Statistical analysis

We performed the meta-analysis using the STATA 12.0 software (Stata Corp.; College Station, TX, USA). The odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated under different genetic models. The *Z*-test was used to estimate the statistical significance of ORs. Power calculations were conducted using PS Power and Sample Size Calculations (Dupont and Plummer Jr., 1990). The Cochran *Q*-test and the I^2 test were used to evaluate potential heterogeneity between studies (Zintzaras and Ioannidis, 2005). If the *Q*-test showed a $P < 0.05$ or the I^2 test showed a result >50%, indicating significant heterogeneity, the random-effect model was conducted; otherwise, the fixed-effect model was used. We also performed subgroup and meta-regression analyses to explore potential sources of heterogeneity. Sensitivity analysis was performed by omitting each study in turn to evaluate the influence of

single studies on the overall estimate. Begger's funnel plots and the Egger linear regression test were conducted to investigate publication bias (Peters et al., 2006).

RESULTS

Characteristics of the studies included

A total of 115 articles relevant to the searched key words were initially identified. The titles and abstracts of all articles were reviewed and 53 were excluded; full texts and data integrity were then reviewed and another 51 papers were excluded. A total of 11 case-control studies were included in this meta-analysis (Roberts et al., 2008; Amre et al., 2009; Dema et al., 2009; Latiano et al., 2009; Meggyesi et al., 2010; Wolfkamp et al., 2010; Prager et al., 2012; Wang et al., 2012; Zheng and Pang, 2012; Durães et al., 2013; Moon et al., 2013). Publication years of the eligible studies ranged from 2008-2013. The selection process of the eligible studies is shown in Figure 1. The distribution of the number of topic-related studies in the electronic database for the last decade is shown in Figure 2. A total of 10,754 subjects were involved in this meta-analysis, including 5183 CD patients and 5571 healthy controls. Three common SNPs (rs13361189 C>T, rs10065172 C>T, and rs4958847 A>G) in the *IRGM* gene were assessed. All powers for the sample size of the studies included were higher than 0.70. Nine studies were conducted in Caucasian populations and only 2 studies were conducted in Asian populations. The TaqMan assay method was performed in 8 studies; the other 3 studies used direct sequencing methods, the polymerase chain reaction-restriction fragment length polymorphism method, and the MassArray method. SNP frequencies of healthy controls were all in HWE (all $P > 0.05$). NOS scores of all studies included were ≥6 (moderate-high quality). The study characteristics and methodological quality are summarized in Table 1.

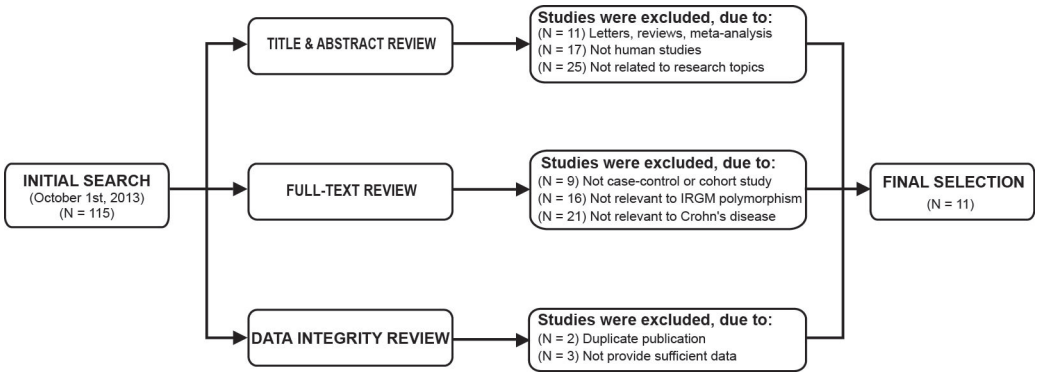

Figure 1. Flow chart of literature search and study selection.

Quantitative data synthesis

A summary of the meta-analysis findings regarding the relationships of *IRGM* genetic polymorphisms and susceptibility to CD is shown in Table 2. The random-effect model was conducted because significant heterogeneity existed between studies. Our meta-analysis re-

sults revealed that the *IRGM* rs13361189 polymorphism was significantly associated with an increased risk of CD (C allele *vs* T allele: OR = 1.30, 95%CI = 1.05-1.61, P = 0.017; CC + CT *vs* TT: OR = 1.32, 95%CI = 1.06-1.64, P = 0.013). However, we observed no correlations of rs10065172 and rs4958847 polymorphisms in the *IRGM* gene with susceptibility to CD (all P > 0.05; Figure 3A).

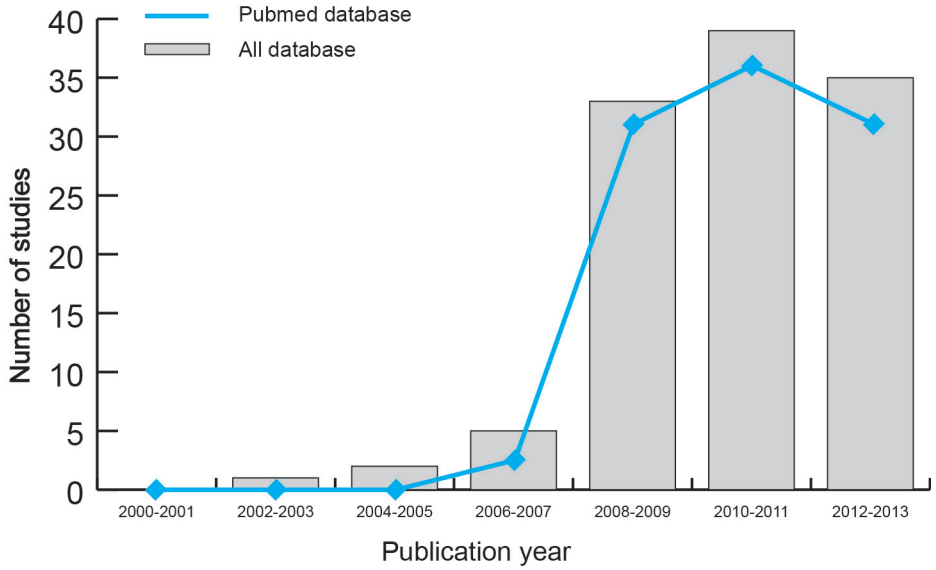

Figure 2. Distribution of the number of topic-related studies in the electronic database for the last decade.

Subgroup analysis by ethnicity suggested significant associations between *IRGM* genetic polymorphisms and an increased risk of CD among Caucasian populations (C allele *vs* T allele: OR = 1.22, 95%CI = 1.07-1.40, P = 0.004; CC + CT *vs* TT: OR = 1.22, 95%CI = 1.05-1.41, P = 0.009), but not among Asian populations (all P > 0.05; Figure 3B). Further subgroup analysis by genotyping method indicated that *IRGM* genetic polymorphisms were significantly associated with the susceptibility to CD in both the TaqMan assay subgroup (C allele *vs* T allele: OR = 1.17, 95%CI = 1.02-1.35, P = 0.030; CC + CT *vs* TT: OR = 1.19, 95%CI = 1.00-1.42, P = 0.046) and non-TaqMan assay subgroup (C allele *vs* T allele: OR = 1.25, 95%CI = 1.01-1.55, P = 0.041; CC + CT *vs* TT: OR = 1.25, 95%CI = 1.03-1.52, P = 0.023; Figure 3C). Further stratification analysis based on sample size showed that *IRGM* genetic polymorphisms may contribute to the susceptibility to CD in the large sample size subgroup (C allele *vs* T allele: OR = 1.31, 95%CI = 1.06-1.62, P = 0.011; CC + CT *vs* TT: OR = 1.34, 95%CI = 1.06-1.69, P = 0.016), while no correlations were observed between *IRGM* genetic variants and CD risk in the small sample size subgroup (all P > 0.05; Figure 3D).

Univariate and multivariate meta-regression analyses showed that ethnicity may be the main source of heterogeneity (P = 0.003; Table 3). The results of sensitivity analysis suggested that no single study could influence the overall pooled ORs (Figure 4). We found no evidence of obvious asymmetry in the Begger's funnel plots (Figure 5). The Egger test also did not display strong statistical evidence of publication bias (C allele *vs* T allele: t = -2.60, P = 0.019; CC + CT *vs* TT: t = -1.54, P = 0.141).

Table 1. Baseline characteristics and methodological quality of all studies included.

First author	Year	Country	Ethnicity	Sample size			Gender (M/F)		Age (years)		Genotyping method	SNP type	NOS score
				Case	Control	Power	Case	Control	Case	Control			
Moon CM	2013	Korea	Asian	253	520	0.777	159/94	294/226	25.9 ± 10.4	39.3 ± 15.8	TaqMan	rs10065172/rs4958847	8
Duraes C	2013	Portugal	Caucasian	511	626	0.814	236/275	241/385	28.6 ± 11.2	30.5 (9-83)	TaqMan	rs13361189	8
Zheng LM	2012	China	Asian	318	318	0.764	154/164	156/162	37.2 ± 11.4	36.7 ± 12.3	Direct sequencing	rs13361189	8
Wang MH	2012	America	Caucasian	227	201	0.743	78/149	86/115	26.7 ± 12.9	-	TaqMan	rs4958847/rs13361189/ rs10065172	7
Prager M	2012	Germany	Caucasian	464	508	0.797	174/290	295/213	29.5 ± 11.6	60.0 ± 16.2	TaqMan	rs4958847/rs13361189	7
Wolfkamp SC	2010	Netherlands	Caucasian	256	529	0.779	-	-	-	-	PCR-RFLP	rs4958847/rs13361189	6
Meggyesi N	2010	Hungary	Caucasian	810	469	0.828	434/376	251/218	37.1 ± 12.6	40.5 ± 11.5	TaqMan	rs13361189	8
Latiano A	2009	Italy	Caucasian	823	578	0.840	468/355	-	30 ± 15	-	TaqMan	rs4958847	6
Dema B	2009	Spain	Caucasian	725	956	0.868	-	-	-	-	TaqMan	rs10065172/rs4958847	6
Amre DK	2009	Canada	Caucasian	289	290	0.758	160/126	125/138	12.1 ± 3.5	11.4 ± 6.8	MassArray	rs10065172	7
Roberts RL	2008	New Zealand	Caucasian	507	576	0.808	-	236/340	-	-	TaqMan	rs13361189/rs4958847	6

M = male; F = female; SNP = single nucleotide polymorphism; NOS = the Newcastle-Ottawa Scale; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.

Table 2. Meta-analysis of the association between *IRGM* genetic polymorphisms and Crohn's disease risk.

Subgroup analysis	W allele *vs* M (Allele model)			WM + MM *vs* WW (Dominant model)			MM *vs* WW + WM (Recessive model)			MM *vs* WW (Homozygous model)			MM *vs* WM (Heterozygous model)		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Overall	1.19	1.06-1.34	0.003	1.21	1.06-1.38	0.005	1.24	1.00-1.54	0.055	1.33	1.04-1.72	0.026	1.12	0.93-1.35	0.225
SNP type															
rs10065172 (C>T)	1.09	0.85-1.40	0.477	1.10	0.81-1.49	0.552	1.21	0.87-1.70	0.253	1.29	0.81-2.06	0.278	1.17	0.91-1.51	0.231
rs4958847 (A>G)	1.17	0.97-1.41	0.101	1.18	0.96-1.45	0.112	1.17	0.83-1.64	0.376	1.23	0.83-1.83	0.310	1.06	0.80-1.41	0.693
rs13361189 (C>T)	1.30	1.05-1.61	0.017	1.32	1.06-1.64	0.013	1.61	0.93-2.77	0.087	1.74	0.99-3.06	0.055	1.36	0.82-2.24	0.229
Ethnicity															
Asians	1.08	0.81-1.43	0.591	1.61	0.80-1.68	0.422	1.01	0.63-1.61	0.971	1.10	0.61-1.99	0.744	0.94	0.62-1.44	0.786
Caucasians	1.22	1.07-1.40	0.004	1.22	1.05-1.41	0.009	1.34	1.03-1.75	0.028	1.43	1.06-1.92	0.019	1.19	0.96-1.47	0.112
Genotyping method															
TaqMan assay	1.17	1.02-1.35	0.030	1.19	1.00-1.42	0.046	1.13	0.94-1.37	0.199	1.20	0.94-1.55	0.150	1.07	0.92-1.25	0.370
Non-TaqMan assay	1.25	1.01-1.55	0.041	1.25	1.03-1.52	0.023	1.98	0.88-4.45	0.099	2.08	0.95-4.56	0.066	1.76	0.79-3.93	0.168
Sample size															
Small sample size (N < 1000)	1.12	0.97-1.29	0.115	1.12	0.96-1.31	0.133	1.17	0.88-1.55	0.269	1.25	0.90-1.74	0.187	1.12	0.86-1.45	0.411
Larger sample size (N > 1000)	1.31	1.06-1.62	0.011	1.34	1.06-1.69	0.016	1.38	0.98-1.94	0.066	1.50	1.01-2.23	0.043	1.18	0.88-1.56	0.267

W = wild-type allele; M = mutant allele; WW = wild homozygote; WM = heterozygote; MM = mutant homozygote; SNP = single nucleotide polymorphism.

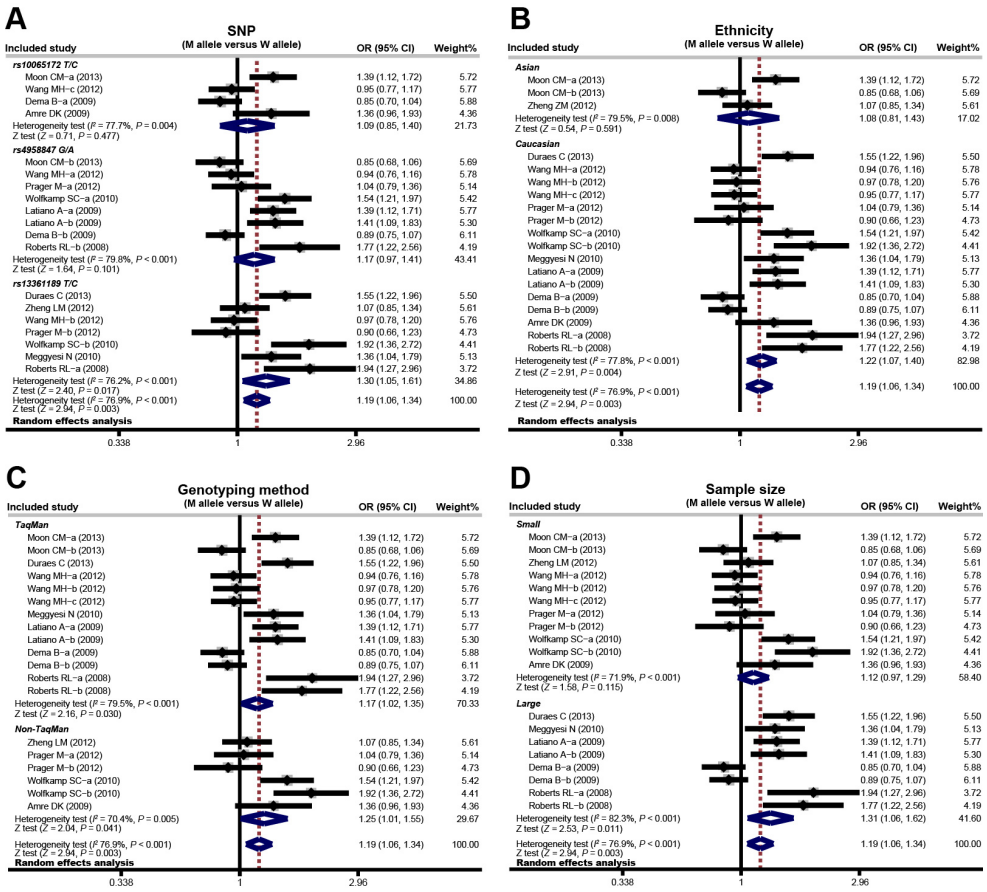

Figure 3. Subgroup analyses of the associations between *IRGM* genetic polymorphisms and CD risk under the allele model.

Table 3. Univariate and multivariate meta-regression analyses of potential sources of heterogeneity.

Heterogeneity factors	Coefficient	SE	*Z*	P	95%CI	
					LL	UL
Publication year						
Univariate	-0.589	0.039	-1.49	0.137	-0.136	0.019
Multivariate	-0.062	0.066	-0.94	0.348	-0.192	0.068
SNP type						
Univariate	0.094	0.091	1.04	0.289	-0.083	0.272
Multivariate	0.097	0.108	0.90	0.369	-0.114	0.308
Ethnicity						
Univariate	-0.258	0.140	-1.84	0.065	-0.532	0.016
Multivariate	-0.534	0.182	-2.94	0.003	-0.890	-0.177
Genotyping method						
Univariate	0.053	0.150	0.35	0.724	-0.240	0.346
Multivariate	0.130	0.227	0.57	0.567	-0.315	0.575
Sample size						
Univariate	0.163	0.136	1.19	0.232	-0.104	0.429
Multivariate	0.143	0.270	0.53	0.595	-0.385	0.672

SE = standard error; 95%CI = 95% confidence interval; LL = lower limit; UL = upper limit.

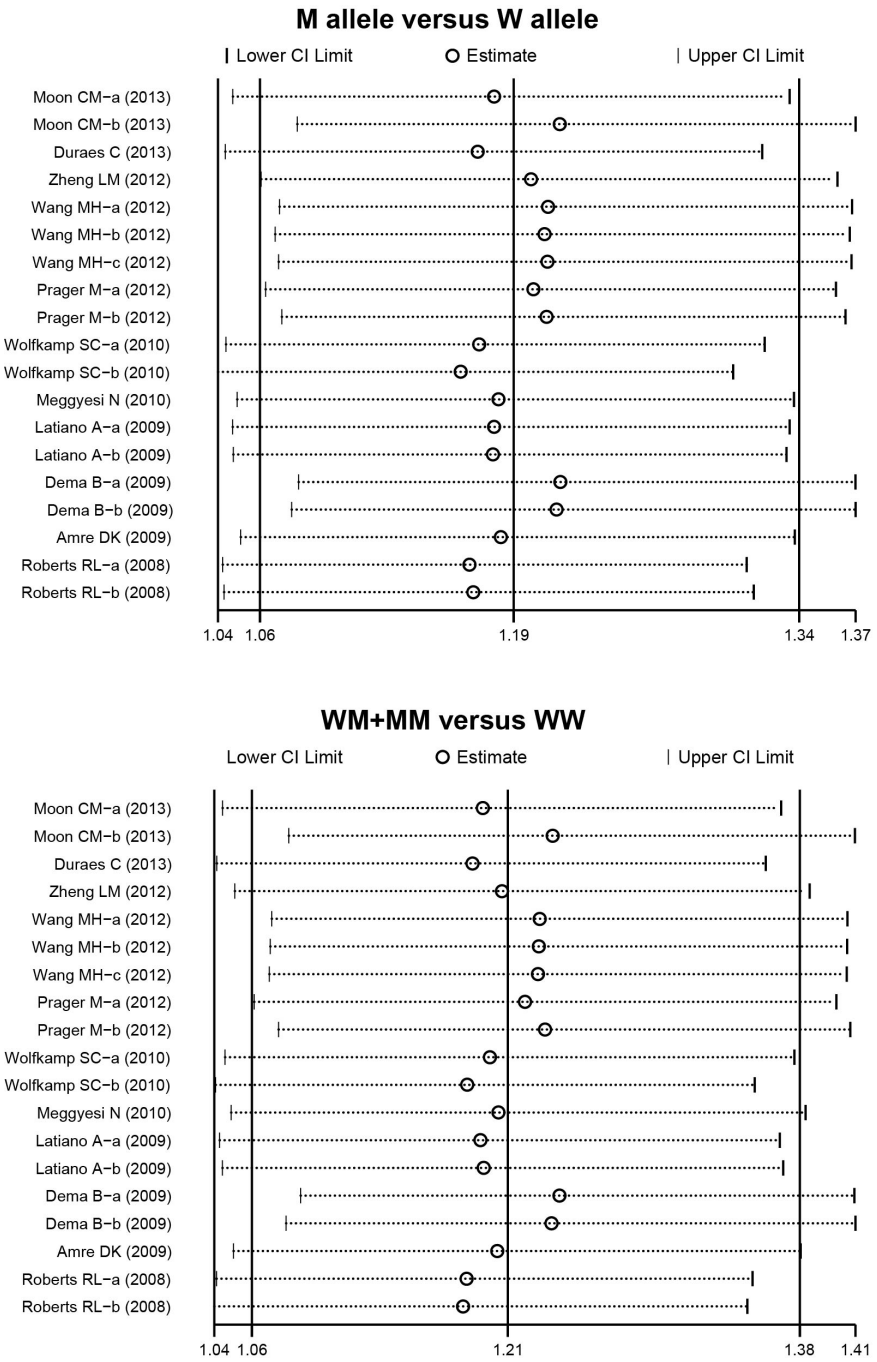

Figure 4. Sensitivity analysis of the summary odds ratio coefficients for the associations between *IRGM* genetic polymorphisms and CD risk under the allele and dominant models. W = wild-type allele; M = mutant allele; WW = wild homozygote; WM = heterozygote; MM = mutant homozygote.

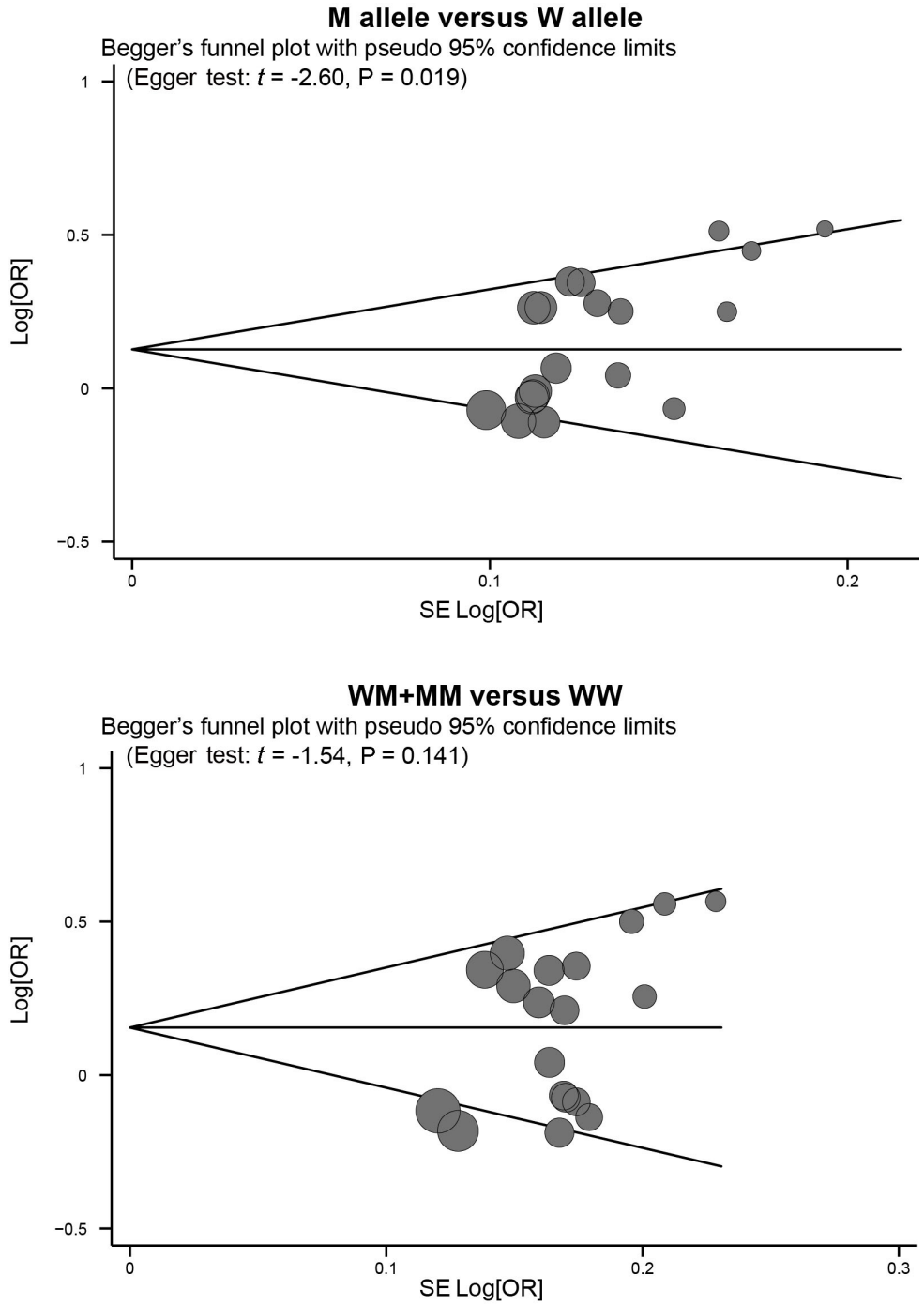

Figure 5. Begger's funnel plots and the Egger linear regression test of publication bias. W = wild-type allele; M = mutant allele; WW = wild homozygote; WM = heterozygote; MM = mutant homozygote.

DISCUSSION

IRGM, a member of the immunity-related GTPases family, refers to a cell-autonomous resistance system that is essential for controlling intracellular pathogens by regulating autophagy formation (Hunn et al., 2011; Lapaquette et al., 2012; Deretic, 2012). IRGM has been indicated to act as a rheostat to fine-tune the autophagy process (Folmes et al., 2012). The regulatory pathways of autophagy integrate a wide range of stress signals that are part of CD physiopathology (Caramés et al., 2010; Deretic et al., 2013). Consequently, IRGM may particularly affect the onset, severity, and relapse of CD (Lapaquette et al., 2010). Several SNPs in the *IRGM* gene such as rs13361189 (C>T), rs10065172 (C>T), and rs4958847 (A>G) have been investigated previously (Waterman et al., 2011; Peter et al., 2011; Moon et al., 2013). Previous studies have shown that these 3 polymorphisms may impact the normal expression of the *IRGM* gene, thus contributing to the susceptibility to CD (Palomino-Morales et al., 2009; Glas et al., 2013).

In the present meta-analysis, we evaluated the relationships between *IRGM* genetic polymorphisms and CD risk. A total of 11 independent case-control studies were included, with a total of 10,754 subjects, including 5183 CD patients and 5571 healthy controls. The results showed that the *IRGM* rs13361189 polymorphism was associated with CD risk, suggesting that the *IRGM* rs13361189 polymorphism may be a risk factor for susceptibility to CD. Although the exact mechanism of *IRGM* genetic polymorphisms in the development of CD are not clear, gene mutation and protein expression of IRGM, as an important chemokine in the pathogenesis of CD, may play a crucial role in the susceptibility to CD (Weersma et al., 2009; Brain et al., 2012). However, there were no associations between *IRGM* rs10065172 and rs4958847 polymorphisms and CD risk, suggesting that these 2 polymorphisms are not determinant factors in the pathogenesis of CD. Since heterogeneity existed in previous individual studies, subgroup analysis was carried out. Our results revealed a relationship between the *IRGM* rs13361189 polymorphism and an increased risk of CD among Caucasians, while similar associations were not observed among Asians. Geographical and ethnic factors may be responsible for individual differences in the susceptibility to CD. In addition, further subgroup analysis based on sample sizes indicated that *IRGM* genetic polymorphisms were related to CD susceptibility in the large sample size subgroup, but not in the small sample size subgroup. There results indicated that sample size is a potential source of heterogeneity. Overall, our results were consistent with those of previous studies in that the relationships between *IRGM* genetic polymorphisms may be strongly linked to the development and progression of CD.

There were some limitations to this meta-analysis. First, our results may not provide sufficient statistical power to estimate the correlation between *IRGM* genetic polymorphisms and CD risk because of the relatively small sample size. Second, a meta-analysis is a retrospective study that may lead to subject selection bias, thereby affecting the reliability of our results. Third, our meta-analysis failed to obtain original data from the studies included, which may limit further evaluation of the potential roles of *IRGM* genetic polymorphisms in the development of CD. Importantly, the inclusion criteria of cases and controls were not well defined in all studies included, which may also have influenced our results.

In conclusion, the results of our meta-analysis suggest that the *IRGM* rs13361189 polymorphism contributes to the susceptibility to CD. Thus, the *IRGM* rs13361189 polymorphism is promising as a biomarker for early diagnosis of CD. However, the *IRGM* rs10065172 and rs4958847 polymorphisms may not be the major determinants of CD risk. Because of the limitations described above, further detailed studies are required to confirm our findings.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We would like to acknowledge the reviewers for their helpful comments on this paper. We would also like to thank our colleagues at the Department of Gastroenterology, People's Hospital of Liaoning Province.

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