



***NLRP3* rs35829419 polymorphism is associated with increased susceptibility to multiple diseases in humans**

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ABSTRACT. Using a meta-analysis framework, we investigated the association between the *NLRP3* rs35829419 polymorphism and increased susceptibility to diverse diseases in humans. Relevant published studies were identified through a comprehensive and systematic electronic search, using the following scientific literature databases: Science Citation Index, the Cochrane Library, PubMed, Embase, CINAHL, Current Contents Index, Chinese Biomedical, the Chinese Journal Full-Text, and the Weipu Journal. Statistical analysis of data extracted from the selected high quality studies was performed using the Version 12.0 STATA software. A total of 13 case-control studies met our stringent inclusion and exclusion criteria for the present meta-analysis. These 13 high quality studies contained relevant information on 7719 patients with various diseases and 7094 healthy controls. Our meta-analysis results showed that the *NLRP3* gene rs35829419 C>A polymorphism was associated with a significantly increased risk of developing multiple diseases in humans under 5 genetic models (all $P < 0.05$). Data stratification and subgroup analysis based on the disease type revealed that rs35829419 C>A carriers displayed a markedly increase susceptibility to leprosy, colorectal cancer, HIV-1 infection,

rheumatoid arthritis, abdominal aortic aneurysms, inflammatory bowel disease, ulcerative colitis, and atopic dermatitis. In summary, our meta-analysis results revealed the first identified strong correlation between the *NLRP3* rs35829419 polymorphism and increased susceptibility to various diseases in humans.

Key words: *NLRP3*; Genetic polymorphism; Leprosy; Colorectal cancer; HIV-1 infection; Rheumatoid arthritis

INTRODUCTION

The NACHT domain, leucine rich repeats (LRR), and pyrin domain (PYD)-containing protein 3 (NLRP3), formerly known as cryopyrin, is a member of the NOD-like receptor (NLR) family (Choi and Ryter, 2014). NLRP3 is the major player in the multi-protein complex known as the inflammasome, and interacts with several adaptor proteins such as apoptosis-associated speck-like protein (ASC), pro-caspase-1, and other proteins that contain caspase activation and recruitment domains (CARD) (Verma et al., 2008). In humans, NLRP3 is encoded by the NOD-like receptor family, pyrin domain containing 3 gene (*NLRP3*), also known as the cold induced auto-inflammatory syndrome 1 (*CIAS1*) gene (Lamkanfi and Kanneganti, 2010). NLRP3 is primarily expressed by dendritic cells and macrophages and is rapidly activated by pathogen-associated molecular patterns such as viral DNAs, peptidoglycans, bacterial lipopolysaccharides and toxins, as well as stress or danger signals such as UV radiation and reactive oxygen species (Wanderer, 2008). The NLRP3 complex shows a strong response to cytidine phosphate-guanosine DNA, ssRNA, and dsRNA in bacteria and viruses, thus detecting microbial pathogens and mounting an immune response (Villani et al., 2009a). The *NLRP3* gene is approximately 32.9 kb in length, contains 9 exons, and maps to the long arm of chromosome 1q44 (Kastbom et al., 2008; Zhang et al., 2011). Within the *NLRP3* gene, approximately 60 single nucleotide polymorphisms (SNPs) have been identified, and the most prevalent polymorphisms among these are rs35829419 (Q705K), rs10754558, rs4612666, rs4925648, and rs10925019 (Schultz et al., 2009), based on the SNP genotype data available from the US National Institute of Mental Health bipolar disorder (NIMH-BP) database dbGaP. In general, *NLRP3* rs35829419 polymorphisms are associated with various inflammatory diseases such as rheumatoid arthritis, Crohn's disease, and celiac disease (Pontillo et al., 2011; Pontillo et al., 2012a; Tan et al., 2013), and the *NLRP3* polymorphism Q705K in particular is tightly linked to autoimmune disorders such as atopic dermatitis and systemic lupus erythematosus (Roberts et al., 2010; Tan et al., 2013) and to neuropathies such as late-onset Alzheimer's disease (Ben Hamad et al., 2012). It is well known that inflammatory diseases are characterized by excessive production of nuclear factor- κ B (NF- κ B)-dependent cytokines such as interleukin (IL)-1 β , and that the *NLRP3* gene polymorphism Q705K is a gain-of-function variant that leads to hyperactive inflammasomes. The heightened activity of inflammasome sowing to *NLRP3* gene polymorphism Q705K likely constitutively cleaves pro-IL-1 β leading to the overproduction of bioactive IL-1 β , which participates in the host response to injury and infection as a pro-inflammatory cytokine (Roberts et al., 2011; Pontillo et al., 2012a). The pathogenic mechanism involving *NLRP3* rs35829419 SNP is dramatically similar in the diverse disease settings discussed above (Roberts et al., 2010; Ben Hamad et al., 2012), and accumulated evidence suggests that the Q705K polymorphism promotes a disease state by causing a hyper-sensitive response to

pathogen-associated molecular patterns and sustained activation of inflammation (Villani et al., 2009b; Kastbom et al., 2013). However, in direct contrast, other studies have shown a protective role for the *NLRP3* polymorphism Q705K; therefore, the clinical relevance of this polymorphism remains uncertain (Hitomi et al., 2009; Ben Hamad et al., 2012). In order to address this issue, we performed a comprehensive meta-analysis to evaluate the association of the *NLRP3* Q705K gene polymorphism with the progression of a variety of human diseases of high clinical value.

MATERIAL AND METHODS

Search strategy

To identify relevant published studies, a comprehensive literature search was conducted without language restriction using the following computerized bibliographic databases: Science Citation Index, the Cochrane Library, PubMed, Embase, CINAHL, Current Contents Index, Chinese Biomedical; the Chinese Journal Full-Text, and the Weipu Journal (last updated search in September 2014). Only literature published in the English and Chinese languages were selected for this meta-analysis. The highly sensitive search strategy included the search terms: (“*NLRP3* protein, human” or “*NLRP3*” or “PYRIN-containing Apaf1-like protein 1” or “NLR family, pyrin domain containing 3” or “PYPAF1” or “CIAS1” or “cryopyrin”) and (“polymorphism, genetic” or “polymorphism” or “polymorphisms” or “variants” or “SNP” or “mutation” or “genetic variants”). Cross-references of relevant articles were manually searched to identify other relevant studies.

Inclusion and exclusion criteria

The retrieved studies were assessed for their suitability to this meta-analysis using the following inclusion criteria: 1) case-control studies reported the correlation between the single nucleotide polymorphism (SNP) in *NLRP3* (rs35829419) and the risk of human diseases; 2) blood samples were obtained from patients with confirmed diagnosis for each disease type; 3) the reports contained sufficient original data and information on the *NLRP3* rs35829419 polymorphism; 4) distributions of *NLRP3* genotype frequencies met Hardy-Weinberg equilibrium (HWE) in the controls; and 5) only the latest complete study was considered when the extracted studies were published by the same authors. Studies that did not meet the inclusion criteria were excluded, along with studies published in non-English or non-Chinese language.

Study quality and data extraction

The Newcastle-Ottawa Scale (NOS) criteria was employed to assess the methodological quality of the included trials (Zintzaras and Ioannidis, 2005). The assessment of study suitability and quality was performed by two reviewers based on the inclusion/exclusion criteria. A standardized data form was utilized to collect the descriptive information in each included study. Collected information included the first author, publication year or submission year, country, journal, ethnicity, language, diseases type, source of controls, confirmation of diagnosis, and demographic variables of subjects, genotype frequencies, HWE test, and detection method. Disagreements during data collection were resolved by reexamination of all items and by discussion with a third investigator.

Statistical analysis

STATA statistical software (Version 12.0, Stata Corporation, College Station, TX, USA) was applied for statistical analyses. The effect sizes representing the unadjusted ORs for the presence of the *NLRP3* SNP in patients and controls were calculated. A Z test was conducted to assess the significance of the overall effect size. Pooled ORs were generated for comparisons using the allelic (C allele vs A allele), dominant (CC + CA vs AA), recessive (CC vs CA + AA), homozygous (CC vs AA), and heterozygous (CC vs CA) models. The heterogeneity between all the studies was evaluated with Cochran's Q-statistic ($P < 0.05$ was considered significant) and the I^2 test (0%, no heterogeneity; 100%, maximal heterogeneity). A random effects model was used in instances of significant heterogeneity ($P < 0.05$ or I^2 test exhibited $> 50\%$); otherwise, a fixed effect model was applied (Alhopuro et al., 2009; Kastbom et al., 2010). Additionally, a sensitivity analysis was performed to evaluate whether removal of any single study could influence the overall outcomes. Further, Egger's linear regression test and a funnel plot were applied to assess publication bias (Pontillo et al., 2010; Girardelli et al., 2012).

RESULTS

Description of included studies

A total of 361 potentially eligible articles were identified from the literature. After exclusion of 3 duplicate studies and 219 irrelevant articles, 140 articles remained for further evaluation. Subsequently, 124 articles were excluded, and 16 articles were examined closely for qualitative analysis. This resulted in elimination of 3 studies that had insufficient information; finally, 13 case-control studies were incorporated into this meta-analysis that contained a total of 7719 patients diagnosed with diverse diseases and 7094 healthy controls (Hitomi et al., 2009; Villani et al., 2009a,b; Roberts et al., 2010; Roberts et al., 2011; Ben Hamad et al., 2012; Carlstrom, 2012; Pontillo et al., 2012a,b; Ungerback, 2012; Kastbom et al., 2013; Pontillo et al., 2013; Varghese et al., 2013). The various diseases in the included studies were myocardial infarction, Alzheimer's disease, leprosy, ankylosing spondylitis, colorectal cancer (CRC), HIV-1 infection, systemic lupus erythematosus, malignant mesothelioma, rheumatoid arthritis (RA), abdominal aortic aneurysms (AAA), inflammatory bowel disease (IBD), ulcerative colitis (UC), Crohn's disease (CD), type 1 diabetes, celiac disease, and atopic dermatitis. Among the 13 studies, 1 study each was performed in Asian and African populations, and the remaining 11 studies were conducted in Caucasian populations. The healthy controls were drawn from population-based sources. The detection techniques employed in the studies included in this meta-analysis were polymerase chain reaction/ligase detection reaction (PCR-LDR) ($N = 1$) and TaqMan assay ($N = 12$). The baseline characteristics of the 13 studies are shown in Table 1.

Quantitative data synthesis

In this meta-analysis, most of the studies shown in the bivariate boxplot figure fell into the central area of the shaded section, but two studies fell outside this area, indicating heterogeneity among the studies (Figure 1). We then investigated the associations between the allele and genotype frequencies of the *NLRP3* rs35829419 polymorphism and the susceptibilities to related

diseases. The meta-analysis revealed that, out of the 16 diseases reported in the selected studies, the *NLRP3* gene (rs35829419) polymorphism conferred significantly increased susceptibility to 12, in both the allelic and dominant models (allele model: OR = 3.74, 95%CI = 1.91-7.30, $P < 0.001$; dominant allele: OR = 13.37, 95%CI = 3.90-45.82, $P < 0.001$) (Figure 2). The results of this meta-analysis implied that carriers of the *NLRP3* rs35829419 C>A polymorphic variant were at an elevated risk of developing multiple diseases in comparison to human subjects without this variant, based on the pooled OR (all $P < 0.05$) (Table 2).

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

First author	Country	Disease	Sample size		Gender (M/F)		Age (years)		Genotyping method	NOS score
			Patients	Controls	Patients	Controls	Patients	Controls		
Varghese (2013)	Sweden	Myocardial infarction	491	911	349/142	653/258	-	-	TaqMan assay	7
Tan (2013)	China	Alzheimer's disease	1133	1159	6464/669	518/641	79.9 ± 8.1	74.5 ± 6.3	PCR-LDR	8
Pontillo (2013)	Brazil	Leprosy	467	380	325/142	252/128	40.7 ± 17.5	35.9 ± 10.7	TaqMan assay	7
Kastbom (2013)	Sweden	Ankylosing spondylitis	492	793	338/154	396/397	51	54	TaqMan assay	7
Ungerback (2012)	Sweden	Colorectal cancer	348	806	-	-	-	-	TaqMan assay	6
Pontillo (2012)	Brazil	HIV-1 infection	150	158	80/70	72/86	34.0 ± 12.4	29.0 ± 7.8	TaqMan assay	6
Pontillo (2012)	Brazil	Systemic lupus erythematosus	144	158	8/136	83/75	37.0 ± 11.9	34.7 ± 11.7	TaqMan assay	6
Girardelli (2012)	Italy	Malignant mesothelioma	134	256	112/22	118/138	66.0 ± 13.0	42.0 ± 7.0	TaqMan assay	6
Ben Hamad (2012)	France	Rheumatoid arthritis	100	100	-	-	32.0 ± 10.0	-	TaqMan assay	6
	Tunisia	Rheumatoid arthritis	141	191	-	58/133	39.5 ± 20.0	43	TaqMan assay	6
Roberts (2011)	New Zealand	Abdominal aortic aneurysms	1238	731	987/251	469/262	74.2 ± 7.9	69.9 ± 6.7	TaqMan assay	8
Roberts (2010)	New Zealand	Inflammatory bowel disease	982	517	410/572	226/291	-	49 (37-65)	TaqMan assay	8
	New Zealand	Ulcerative colitis	475	517	228/247	226/291	39 (28-53)	49 (37-65)	TaqMan assay	7
	New Zealand	Crohn's disease	507	517	182/325	226/291	32 (23-50)	49 (37-65)	TaqMan assay	7
Pontillo (2010)	Brazil	Type-1 diabetes	196	192	94/102	90/102	7.3 ± 3.4	7.1 ± 4.8	TaqMan assay	6
	Brazil	Celiac disease	59	192	21/38	90/102	10.0 ± 4.0	7.1 ± 4.8	TaqMan assay	6
	Brazil	Atopic dermatitis	165	192	84/81	90/102	7.0 ± 5.0	7.1 ± 4.8	TaqMan assay	6
Schultz (2009)	Sweden	Crohn's disease	497	742	257/244	384/356	28 (10-90)	-	TaqMan assay	7

M = male; F = female; NOS = Newcastle-Ottawa scale; PCR-LDR = polymerase chain reaction-ligase detection reaction.

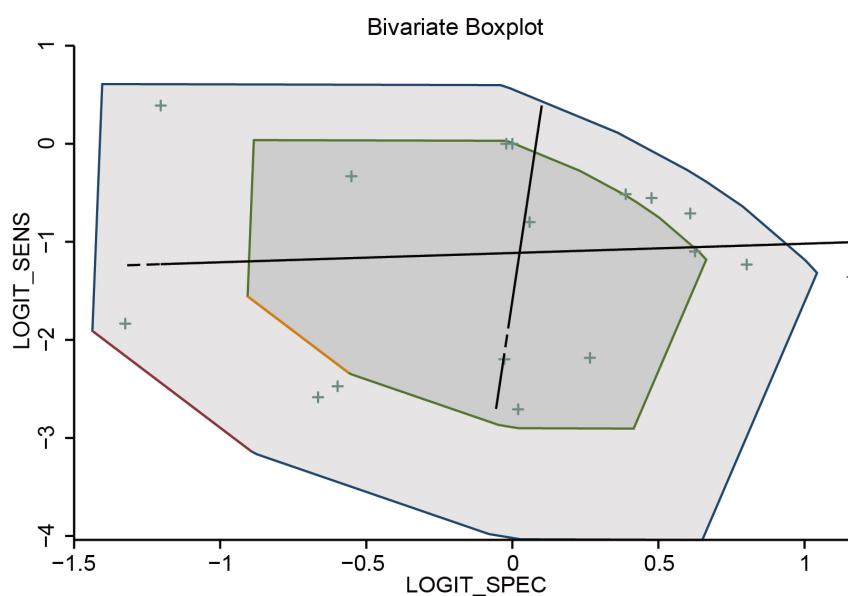


Figure 1. Test for heterogeneity in the included studies by a bivariate boxplot.

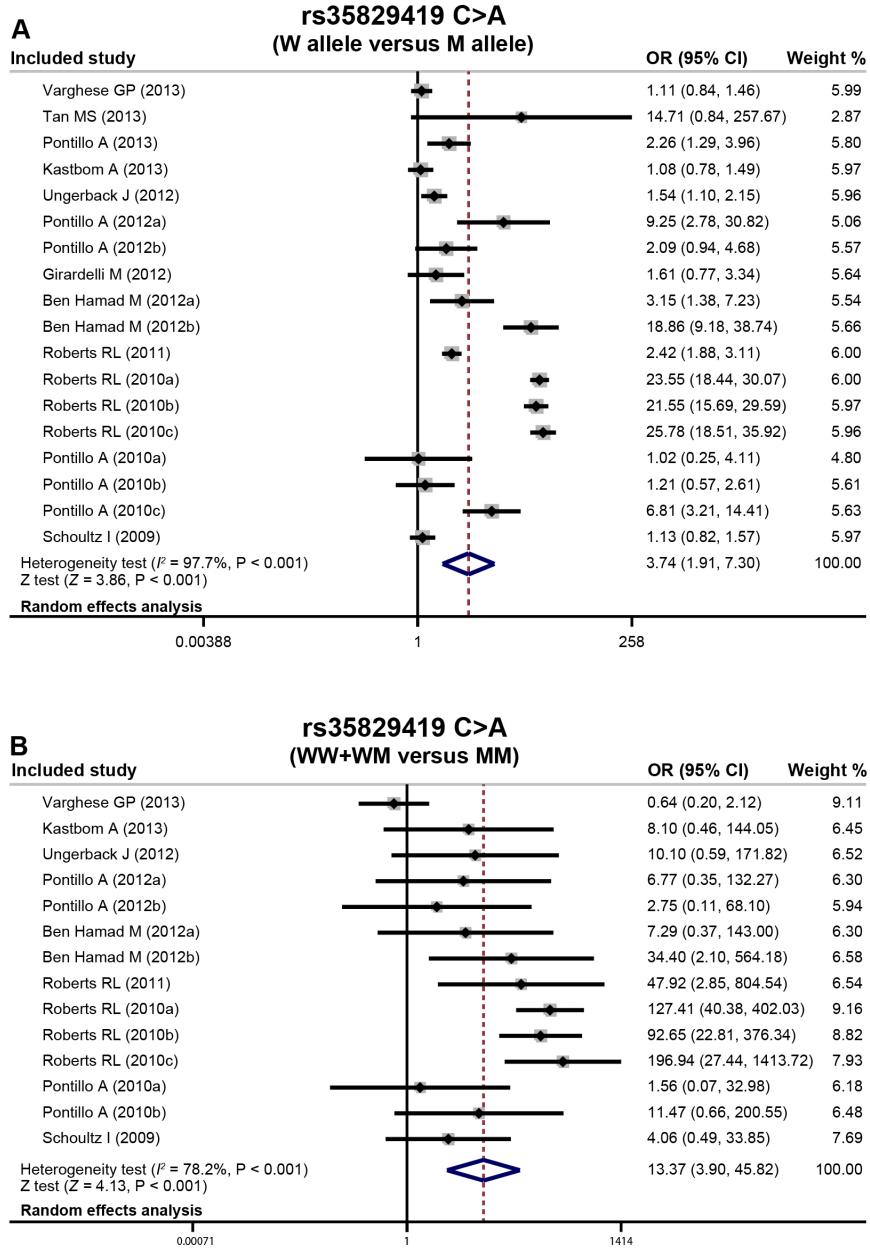


Figure 2. Forest plots for the influences of the *NLRP3* genetic polymorphism and various human diseases under the allelic and dominant models (A, W allele vs M allele; B, WW+WM vs MM).

In subgroup analysis stratified by disease type, the results showed an elevated risk of related diseases in carriers of the *NLRP3* rs35829419 C>A polymorphism in leprosy, CRC, RA, AAA, IBD, UC, and AD in the majority of subgroups (Figure 3).

Table 2. Meta-analysis of the association between the NLRP3rs35829419 C>A polymorphism and various human diseases.

Subgroup analysis	C vs A allele (Allelic model)			CC + CA vs AA (Dominant model)			CC vs CA + AA (Recessive model)			CC vs AA (Homozygous model)			CC vs CA (Heterozygous model)		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Overall	3.74	1.91-7.30	<0.001	13.37	3.90-45.82	<0.001	4.04	1.93-8.44	<0.001	18.79	4.27-82.65	<0.001	3.53	1.83-6.82	<0.001
Country															
Sweden	1.19	1.02-1.39	0.032	2.62	0.60-11.49	0.201	1.16	0.99-1.37	0.073	2.66	0.61-11.58	0.192	1.13	0.95-1.33	0.167
China	14.71	0.84-257.67	0.066	-	-	-	14.75	0.84-258.61	0.065	-	-	-	14.75	0.84-258.61	0.065
Brazil	2.68	1.43-5.04	0.002	4.49	0.99-20.28	0.051	2.66	1.40-5.05	0.003	5.10	1.13-23.07	0.034	2.49	1.36-4.57	0.003
Italy	1.61	0.77-3.34	0.204	-	-	-	1.65	0.78-3.48	0.192	-	-	-	1.65	0.78-3.48	0.192
France	3.15	1.38-7.23	0.007	7.29	0.37-143.00	0.191	2.91	1.22-6.97	0.016	8.14	0.41-160.08	0.168	2.47	1.01-6.04	0.047
Tunisia	18.86	9.18-38.74	<0.001	34.40	2.10-564.18	0.013	25.24	11.98-53.19	<0.001	72.82	4.43-1197.91	0.003	20.75	9.82-43.84	<0.001
New Zealand	13.31	4.01-44.20	<0.001	114.73	52.66-249.93	<0.001	17.99	4.02-80.46	<0.001	309.20	141.26-676.81	<0.001	12.85	3.38-48.75	<0.001
Disease															
Myocardial infarction	1.11	0.84-1.46	0.465	0.64	0.20-2.12	0.470	1.15	0.86-1.55	0.341	0.66	0.20-2.18	0.499	1.19	0.88-1.60	0.265
Alzheimer's disease	14.71	0.84-257.67	0.066	-	-	-	14.75	0.84-258.61	0.065	-	-	-	14.75	0.84-258.61	0.065
Leprosy	2.26	1.29-3.96	0.005	-	-	-	2.31	1.30-4.09	0.004	-	-	-	2.31	1.30-4.09	0.004
Ankylosing spondylitis	1.08	0.78-1.49	0.647	8.10	0.46-144.05	0.154	1.01	0.72-1.43	0.932	8.05	0.45-143.28	0.156	0.95	0.68-1.35	0.788
Colorectal cancer	1.54	1.10-2.15	0.013	10.10	0.59-171.82	0.110	1.47	1.03-2.10	0.034	10.60	0.62-180.44	0.103	1.36	0.95-1.95	0.091
HIV-1 infection	9.25	2.78-30.82	<0.001	6.77	0.35-132.27	0.207	8.78	2.58-29.81	<0.001	7.68	0.39-149.99	0.179	7.68	2.24-26.33	0.001
Systemic lupus erythematosus	2.09	0.94-4.68	0.071	2.75	0.11-68.10	0.536	2.05	0.90-4.69	0.089	2.91	0.12-72.16	0.514	1.94	0.84-4.47	0.119
Malignant mesothelioma	1.61	0.77-3.34	0.204	-	-	-	1.65	0.78-3.48	0.192	-	-	-	1.65	0.78-3.48	0.192
Rheumatoid arthritis	7.81	1.35-45.03	0.022	16.62	2.16-127.57	0.007	8.68	1.05-72.06	0.045	25.89	3.04-220.78	0.003	7.27	0.90-58.41	0.062
Abdominal aortic aneurysms	2.42	1.88-3.11	<0.001	47.92	2.85-804.54	0.007	2.33	1.78-3.05	<0.001	53.22	3.17-893.95	0.006	2.10	1.60-2.76	<0.001
Inflammatory bowel disease	23.55	18.44-30.07	<0.001	127.41	40.38-402.03	<0.001	35.71	22.74-47.51	<0.001	370.41	116.26-1180.12	<0.001	23.61	17.28-32.25	<0.001
Ulcerative colitis	21.55	15.69-29.59	<0.001	92.65	22.81-376.34	<0.001	32.87	22.74-47.51	<0.001	267.63	65.35-1096.00	<0.001	21.95	15.00-32.12	<0.001
Crohn's disease	5.41	0.25-115.45	0.280	28.87	0.64-1294.66	0.083	6.49	0.20-215.67	0.295	49.24	0.39-6293.28	0.115	5.13	0.22-117.70	0.307
Type-1 diabetes	1.02	0.25-4.11	0.977	-	-	-	1.02	0.25-4.14	0.976	-	-	-	1.02	0.25-4.14	0.976
Celiac disease	1.21	0.57-2.61	0.618	1.56	0.07-32.98	0.775	1.15	0.52-2.57	0.728	1.58	0.07-33.52	0.768	1.08	0.48-2.43	0.846
Atopic dermatitis	6.81	3.21-14.41	<0.001	11.47	0.66-200.55	0.095	7.08	3.26-15.40	<0.001	15.29	0.87-267.88	0.062	6.18	2.83-13.53	<0.001

W = wild-type allele; M = mutant allele; WW = wild-type homozygote; WM = heterozygote; MM = mutant homozygote.

Meta-regression analysis results confirmed that no single factor was the main source of heterogeneity (Figure 4 and Table 3). Sensitivity analysis was conducted to determine whether the study conclusions were affected by any single study; the finding suggested that no single study had an effect on the pooled ORs (Figure 5). Furthermore, the Egger's regression test and funnel plot showed symmetrical distribution for the associations of the *NLRP3* rs35829419 C>A polymorphism and the risk of various diseases (all $P > 0.05$) (Figure 6).

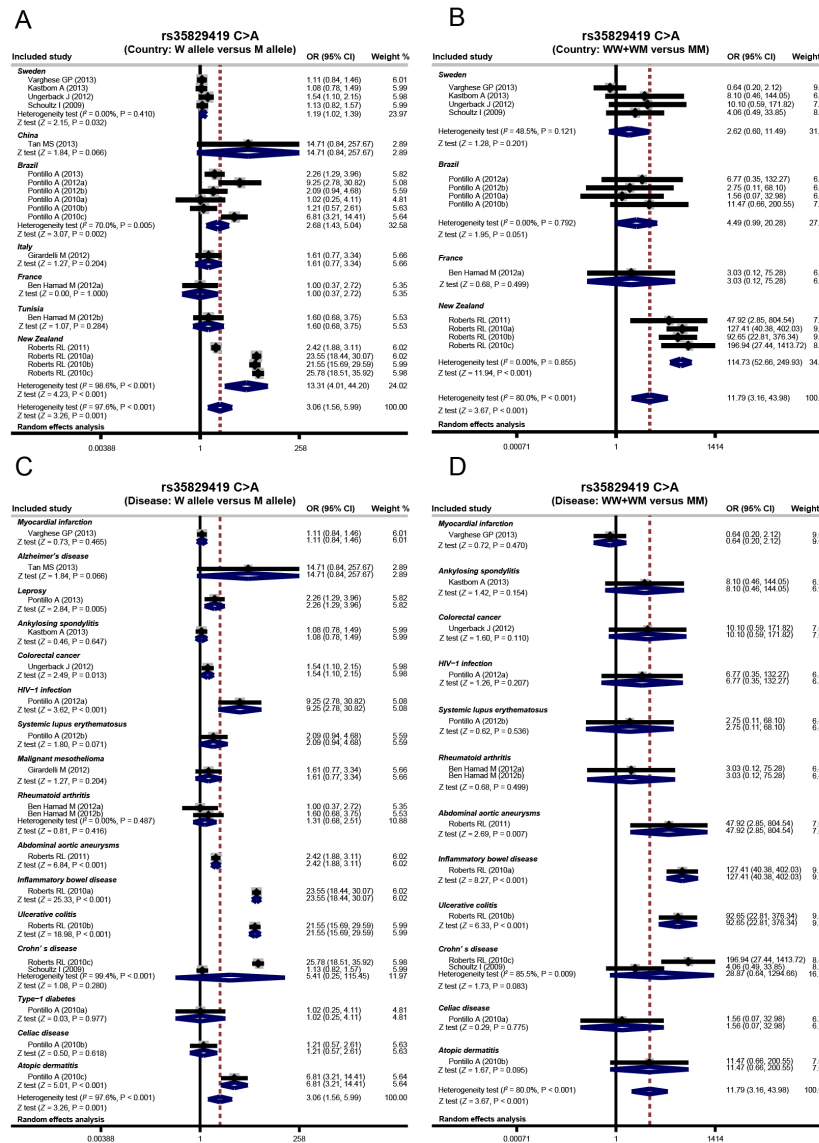


Figure 3. Subgroup analyses for the influences of the *NLRP3* genetic polymorphism and various human diseases under the allelic and dominant models (A, country: W allele vs M allele; B, country: WW+WM vs MM; C, disease: W allele vs M allele; D, disease: WW+WM vs MM).

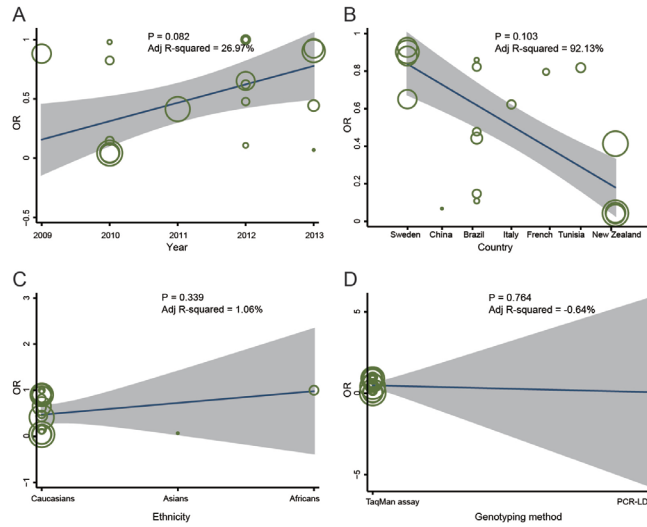


Figure 4. Univariate meta-regression analysis of potential sources of heterogeneity. PCR-LDR, polymerase chain reaction-ligation detection reaction. (A, year; B, country, C, ethnicity, D, genotyping method).

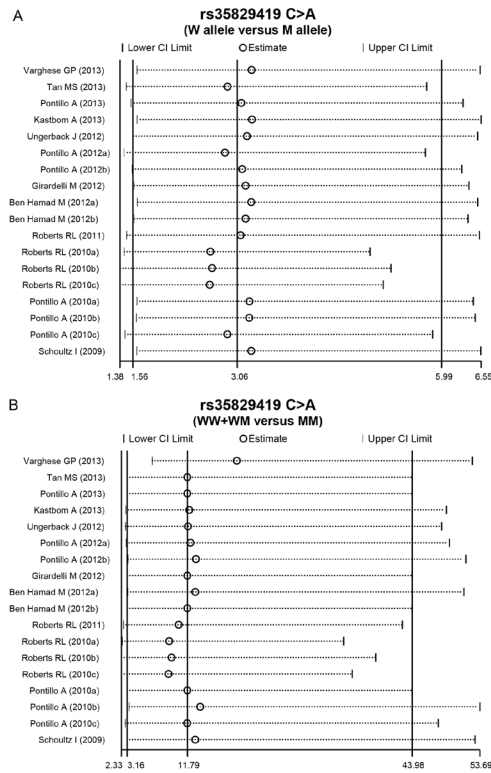


Figure 5. Sensitivity analyses for the influences of the *NLRP3* genetic polymorphism and various human diseases under the allelic and dominant models (A, W allele vs M allele; B, WW+WM vs MM).

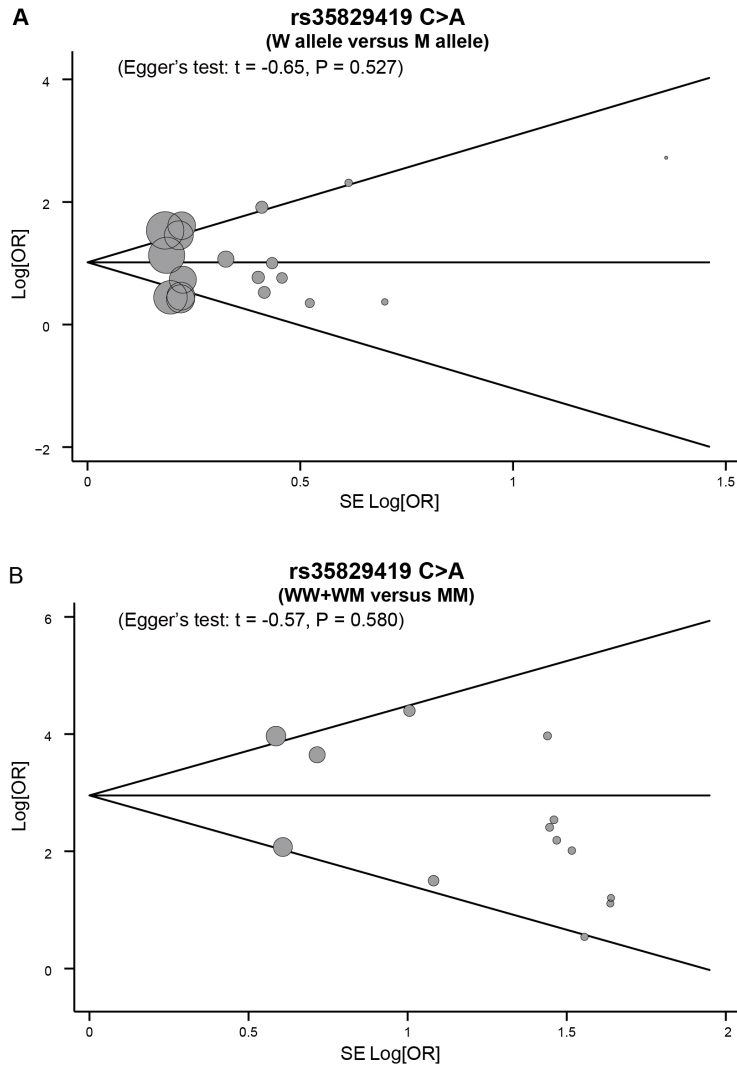


Figure 6. Egger's regression test of the relationships between the *NLRP3* genetic polymorphism and various human diseases under the allelic and dominant models (A, W allele vs M allele; B, WW+WM vs MM).

Table 3. Meta-regression analysis of potential sources of heterogeneity.

Heterogeneity factor	Coefficient	SE	t	P (adjusted)	95%CI	
					LL	UL
Year	0.031	0.049	0.65	0.939	-0.072	0.135
Country	-0.105	0.023	-4.48	0.108	-0.155	-0.054
Ethnicity	0.345	0.186	1.86	0.258	-0.057	0.746
Genotyping method	-1.038	1.478	-0.70	0.910	-4.226	2.150

SE = standard error; LL = lower limit; UL = upper limit.

DISCUSSION

We used a meta-analysis based approach to examine the association between the *NLRP3* rs35829419 gene polymorphism and the development of inflammatory diseases. The results of our meta-analysis suggest a close link between the *NLRP3* rs35829419 gene polymorphism and the disease pathogenesis in leprosy, CRC, RA, AAA, IBD, UC, and AD.

The NLRP3 protein contains NACHT, LRR, and PYD domains. The NACHT domain is a nucleotide binding domain, while LRR is a leucine-rich repeat domain used for detecting microbial pathogens, and PYD is useful for protein-protein interaction with the PYD of apoptosis-associated speck-like protein (ASC), which also contains a CARD domain; together these domains are important in assembly of the inflammasome multi-protein complex for mediating the inflammatory process and apoptosis signaling in response to various stimuli (Ji et al., 2012). Through PYD-PYD interactions, NLRP3 recruits ASC, which in turn leads to the assembly of pro-caspase-1 through CARD-CARD interactions, and its subsequent activation allows the maturation and release of IL-1 β , causing widespread inflammatory reactions (Cummings et al., 2010).

NLRP3 gene polymorphisms are associated with various inflammatory diseases such as CRC, AAA, and CD (Hitomi et al., 2009). The rs35829419 variant is located in exon 3 of the *NLRP3* gene, which is close to the NACHT domain where most *NLRP3* gene mutations are also found (Pontillo et al., 2010). In this meta-analysis, we determined that the minor A allele of rs35829419 conferred protection against CRC, the mechanisms of which might be related to the interaction of NLRP3 with caspase-1 in inflammasomes, promoting release of pro-inflammatory cytokines such as IL-1 β through the inflammatory NF- κ B pathway, which participates in apoptosis (Varghese et al., 2013). On the other hand, in IBD, including UC and CD, and in abdominal aortic aneurysms, the protective mechanism is thought to involve a genetic interaction between the rs2043211 polymorphism in the *CARD8* gene and rs35829419 in *NLRP3* (Villani et al., 2009b). The T allele in rs2043211 of the *CARD8* gene produces a severely truncated *CARD8* protein; as *CARD8* is a constituent protein of the NLRP3 inflammasome, *CARD8* truncation further results in promoting pro-IL-1 β production and there by preventing the pathological inflammation typical in IBD (Roberts et al., 2011). Based on previous studies and our results in this meta-analysis, we propose that the *NLRP3* gene rs35829419 polymorphism affects a variety of inflammatory diseases, positively or negatively, primarily through its role in the inflammasome complex and its protein-protein interactions with other members of the complex. Similar conclusions were reached by Varghese et al. (2013), who showed that the minor A allele in the rs35829419 polymorphism was protective against myocardial infarction in women through increased production of IL-1 β , which had a cooperative beneficial effect in combination with the female hormone estrogen (Carlstrom, 2012).

In order to investigate other influencing factors, subgroup analysis based on country showed a strong link in both the allelic and dominant models between the *NLRP3* gene polymorphism and the studied diseases in New Zealand and Tunisian populations. However, in other countries the variants had no detectable disease associations, which might be due to environment, genetic background, or lifestyle differences. Clear relationships between rs35829419 and RA, IBD, UC, and AAA were observed in both the allelic and dominant models. Our results are partly in agreement with previous studies in that the *NLRP3* gene rs35829419 polymorphism was found to be linked to several inflammatory diseases, suggesting that the *NLRP3* genetic polymorphism is a critical predisposing factor in the development of various human diseases. Therefore, detection of

the *NLRP3* rs35829419 gene polymorphism might permit early prediction of the risks for various human diseases, and might provide a basis for designing treatments as well as for additional studies on the susceptibility to disease.

The limitations of our study should be also mentioned. First, only one SNP (rs35829419) was investigated, and the involvement of additional genomic loci in the disease outcomes cannot be ruled out. Additionally, there were a total of 16 different types of diseases involved in the present meta-analysis that might be caused by different etiological factors; therefore, the clinical applications of our final results might be limited. Thirdly, the current meta-analysis only included trials published in English and Chinese, and nearly all (11/13) of the studies were performed in Caucasians with only 1 in Asians and 1 in Africans. Further studies are needed to better represent other ethnic groups to clarify any ethnic differences in the frequency of effect of the *NLRP3* rs35829419 polymorphism. Furthermore, heterogeneity existed in this meta-analysis and complete data was not available from all of the included studies. The study in Asians did not conform to HWE, which influenced the accuracy of the results, and should have been excluded.

Taken together, our findings suggest that the *NLRP3* rs35829419 genetic polymorphism is involved in the progression of several human diseases, especially of leprosy, CRC, RA, AAA, IBD, UC, and AD. Further clinical studies and functional analyses are required to investigate the mechanisms underlying the genetic interactions between the *NLRP3* gene with other genomic loci, and the susceptibility to various diseases in humans.

Conflicts of interest

The authors declare no conflict of interest.

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