Review

rs10865331 in 2p15 increases susceptibility to ankylosing spondylitis: a HuGE review and meta-analysis

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ABSTRACT

Objective. *2p15 polymorphisms have been reported to increase ankylosing spondylitis (AS) susceptibility in several studies; however, when it comes to whether and how much of this risk exists, the results are inconclusive. The aim of this study is to investigate the correlation between rs10865331 in 2p15 and the risk of AS.*

Methods. *We conducted a HuGE review and meta-analysis of studies published through September 2019. Studies were identified in PubMed, Scopus, HuGE Navigator, Embase, and Web of Science databases. Odds ratios (ORs) and 95% confidence intervals (CIs) for risk estimations were calculated. Sensitivity analysis, subgroup analysis and analysis for potential publication bias were also estimated.*

Results. *Eleven studies with 18555 AS patients and 43777 unrelated healthy individuals, each with a score greater than 6 on the Newcastle-Ottawa Scale (NOS), that investigated the association between rs10865331 in 2p15 and AS were included in our meta-analysis. Data were classified into the genotype analysis cohort, the OR-value cohort, and the pooled analysis cohort, and then a meta-analysis was performed. The OR value of the recessive model in the genotype analysis cohort was 1.376 (95% CI=1.204-1.572, p<0.001, I^2=56.30%), and the OR value of the pooled analysis cohort was 1.295 (95% CI=1.228-1.365, p<0.001, I^2=73.70%). These findings suggest that individual who carries this single nucleotide polymorphism (SNP) are about 30% more susceptible to developing AS.*

Conclusion. *Our results suggest that rs10865331 is associated with a significantly higher risk of AS in all race and country subgroups that we have evaluated. Therefore, rs10865331 may be a useful genetic marker for predicting AS susceptibility. However, further studies are needed to confirm our findings.*

Introduction

Ankylosing spondylitis (AS) is a common and progressive inflammatory rheumatic disease, it is a type of spondyloarthritis (SpA) (1-2). AS primarily leads to destruction in the axial skeleton (3), and the characteristic clinical manifestations of AS are inflammatory back pain and progressive spinal stiffness, extraskeletal lesions, such as acute anterior uveitis and Crohn's disease (4-8), may also occur. The prevalence of AS ranges from 7.4 of 10,000 people in Africa to 23.8 of 10,000 people in Europe (9). Unfortunately, the aetiology and pathogenesis of AS remain unclear (10). Due to the limitations of current treatments (11-12), AS gives rise to social and economic burdens and is associated with a low health-related quality of life (HRQoL) (13-15). Since AS is highly heritable (17-18), genetic factors play a pivotal role in the pathogenesis and development of AS. Increasing evidence suggests that several pathways17 and candidate genes, including endoplasmic reticulum aminopeptidase 1 (ERAP1) and interleukin-23 receptor (IL-23R) (19-24), have been identified as biomarkers of AS. Specifically, human leukocyte antigen-B27 (HLA-B27) is strongly associated with AS and can be detected in most AS patients (25). However, it has also been reported that HLA-B27 only accounts for a minority of the overall genetic susceptibility of AS26-27. Therefore, other factors must also be largely involved (27).

A genome-wide association study (GWAS) was performed in a population of European descent in 2010 (28). The study detected that 2p15 was a

susceptibility area for AS, which was further confirmed in several European and Chinese GWASs (29-31). Among the non-major histocompatibility complex (non-MHC), rs10865331, a single nucleotide polymorphism (SNP) in 2p15, was reported to increase the risk of AS (32), but the functions of this SNP and the area of 2p15 had yet remained unclear. This SNP is present in a gene desert located 99 kb upstream of beta-1,3-N-acetylglucosaminyltransferase 2 (B3GNT2) and 182 kb downstream of transmembrane protein 17 (TMEM17) (33). And the frequency of rs10865331 ranges from 0.357 to 0.59 in globally reported studies (32).

Several case-control studies have investigated if rs10865331 is associated with AS susceptibility, but whether and how much this SNP increases the susceptibility to AS remains to be determined. Therefore, we conducted a HuGE review and meta-analysis to clarify the correlations between rs10865331 and AS susceptibility.

Methods

This HuGE review and meta-analysis were performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyzes (PRISMA) statement (34-35), HuGE Review Handbook ver. 1.0 (36), the Cochrane Handbook for Systematic Reviews of Interventions (37), and our previous study (56). The PRISMA checklist and a checklist of HuGE review are provided in the online Supplementary file.

Literature search

Case-control, cross-sectional, and cohort studies that analysed the relationship between the rs10865331 polymorphism and AS susceptibility were considered for our review and meta-analysis. We comprehensively searched for potential references by computer-based searches from PubMed, Web of Science, Scopus, HuGE Navigator, and Embase databases through September 2019 using key words such as "rs10865331", "2p15", "ankylosing spondylitis". The last literature search was updated to 30th, September 2019. No language limitation was set. Reference list was also comprehensively screened. The detailed literature retrieval strategy is provided in the Supplementary file.

Study selection

This meta-analysis is based on the following three types of studies: 1) published studies about AS risk and rs10865331, 2) studies involving patients with AS only according to diagnosis by imaging, pathology, or the latest clinical diagnostic criteria (38), and genotyping method was based on valid molecular techniques, and 3) studies with detailed patient data or correlation conclusions for quantitative or qualitative analysis. Moreover, only studies with the largest sample size or the latest published studies in the case of the same or overlapping data were chosen, while studies with data missing were just reviewed rather than selected in the meta-analysis.

Data extraction

Two investigators were appointed for data extraction following a standard protocol and another investigator was sent for double-check of data. A senior investigator is needed to reach a consensus for any inconsistent data via an open discussion.

Reference quality assessment

Quality of selected studies was assessed by two investigators with Newcastle-Ottawa Scale (NOS) (39). NOS which is designed for the purpose of case-control studies covers three domains, namely, selection, comparability, and exposure-8 items with a total of 9 stars. A study was considered to be of high quality (or have low-bias risk) and would be used if it achieved a score of 6 to 9 stars. A score of 4 or 5 stars was considered to indicate intermediate-bias risk and 1 to 3 stars indicated high-bias risk.

Statistical analysis

The meta-analysis was accomplished with Stata 11.0 software. A Hardy-Weinberg equilibrium (HWE) test of every control group was carried out based on the collected data, in which a *p*-value of less than 0.05 indicated significant differences. Detailed patient data of every genotype and OR values were used in our meta-analysis. Pooled data would be considered low heterogeneity if *p*-value was greater than 0.1 and I^2 was less than 50%. These cases applied a fixed effects model and the rest cases applied a random effect model (40-42). Moreover, recessive model 22 *versus* 11+12, dominant model 12+22 *versus* 11, and allele model 1 *versus* 2 (where 1 represents the wild allele and 2 represents the mutated allele) were used for the statistical analysis on genotype analysis cohort. In the OR-value cohort, OR value and 95% CI provided in the included articles would be used. And in the pooled analysis cohort, OR values provided in the included articles and crude OR values obtained from genotype analysis were used together, in order for all studies to be included. Statistical analyses were double-sided and a *p*-value less than 0.05 was considered significant. The overall effect was estimated by Z-score which was assessed by the *p*-value of double-sided U-test.

Subgroup analyses

Subgroups were analysed with considerations to demographic or clinical characteristics, aiming to disclose heterogeneity sources and potential correlations.

Heterogeneity and sensitivity analyses

Heterogeneity sources and influences of excluded studies on meta-analysis results were introduced in a sensitivity analysis. In this sensitivity analysis, effects of each study on the final meta-analysis results were investigated by an exclusive method. Studies which induced a reduction of I^2 (even decreased to 0%) after being removed from the meta-analysis were determined a heterogeneity source. Subsequently, meta-analysis results before and after the removal of the screened studies were compared. The maximum and/or minimum extremes caused by significant heterogeneity were deleted for the sake of conservative analysis.

Publication bias

A quantitative assessment on potential publication preference was carried out through Egger's linear regression and Begg's rank correlation tests (43-44).

There is a significant publication preference if a *p*-value less than 0.05. In this study, visual and qualitative analyses on publication preference were accomplished under the assistance of Funnel plots. If publication bias did not exist, the plot resembled a symmetrical inverted funnel. If publication bias was present, the Duval and Tweedie nonparametric trim-and-fill analysis was conducted and Rosenthal's fail-safe number was calculated to account for potential publication bias and estimate the stability of our outcomes (45-47).

Results

Reference search

A total of 74 associated studies were searched by titles, abstract and full text (Fig. 1), we comprehensively searched the databases a second time to avoid omission. Finally, we identified eleven studies that reported associations between rs10865331 in 2p15 and AS susceptibility. Seven studies (29, 48-54), including a case-control GWAS study and six case-control studies of genotype distribution data, were available and included in our final analysis. Four studies (28-30, 55), including a case-control study and three case-control GWAS studies, did not include detailed genotype distribution data and only OR values and 95% CIs were available.

For the studies in which genotype data were available, we performed a meta-analysis with genotype distributions. These studies were grouped as the genotype analysis cohort. For the studies that provided OR values and 95% CIs of A *versus* G, we combined these studies and included them in the OR-value cohort; for the studies without given A *versus* G OR values, we calculated crude OR values with genotype distributions, together with the OR-value cohort, and included them as the pooled analysis cohort. Some included studies provided OR values and 95% CIs in addition to genotype distribution data: these studies were included in all three cohorts.

Data extraction and reference assessment

Only 11 studies were selected in the meta-analysis according to the above-mentioned criteria. Extracted data are displayed in Table I. Seven studies were included in the genotype analysis cohort and eleven OR values and their 95% CIs were included in the OR-value cohort; 16 OR values and 95% CIs were included in the pooled analysis cohort. In all, 6048 AS patients and 8590 control subjects were included in the genotype analysis cohort; 17294 AS patients and 42267 control subjects were included in the OR-value cohort; and 18555 AS patients and 43777 control subjects were included in the pooled analysis cohort.

Data acquisition was finished after overcoming several challenges. The study by Jung *et al*. (44) had two pairs of case-control groups – one that belonged to a scale construction set and one that belonged to an independent validation set; and only OR values and 95% CIs of the dominant model were provided thus abandoned; therefore, we only included this study in the genotype analysis cohort. The study by Lin *et al*. (29) had two case groups and two control groups. The *p*-value of HWE in the study by Davidson *et al*. (54) was not available. But in their study, the author pointed out that if HWE *p*-value of one SNP was less than 0.0005, this SNP would be excluded, thus we inferred that the *p*-value of HWE in this SNP was ≥0.0005 as per this study. In the study by Reveille *et al*. (28), the OR value was not coincident between in-article and Supplementary file, so we used only in-article data.

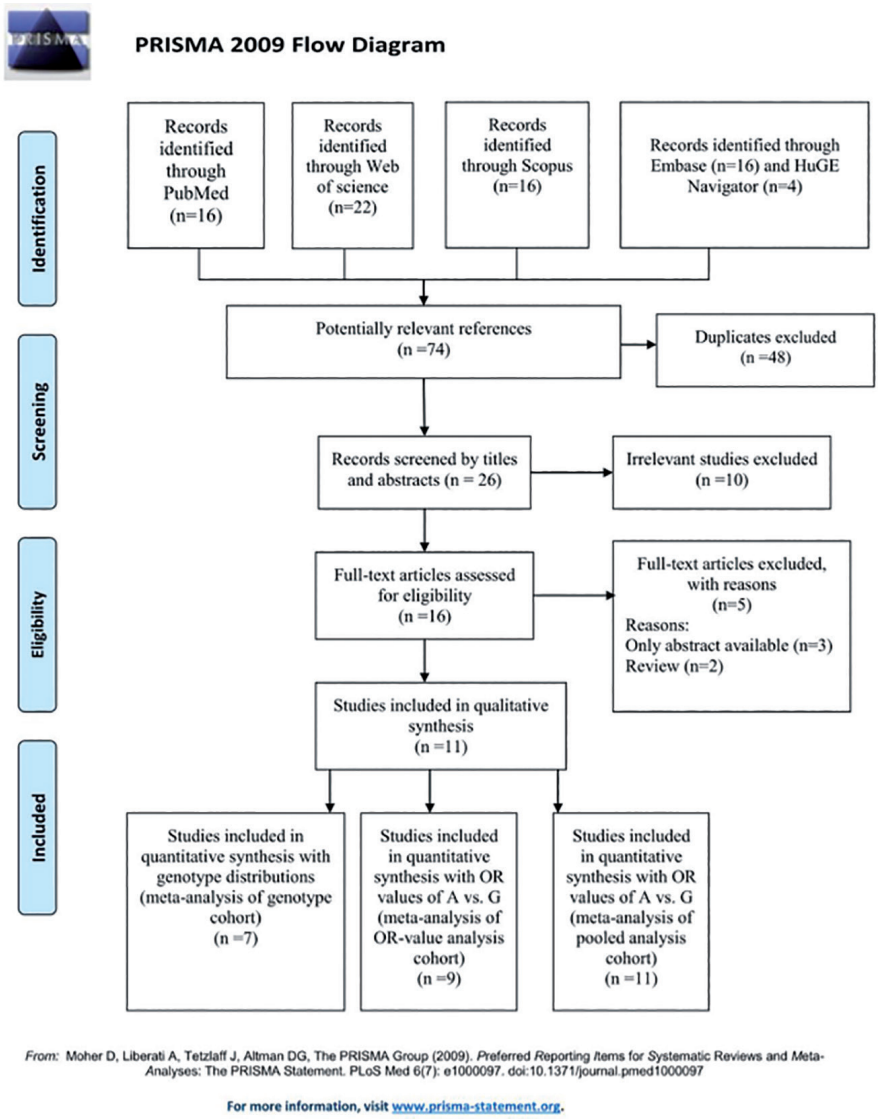

Fig. 1. PRISMA 2009 flow diagram.

Table I. Characteristics of included studies.

First Author	Year	Country	Study design	No. of subjects, HLA-B27(+) %		Gender (F/M)		Ages (year)		Gene distributions (GG/GA/AA)		HWE of control group
				Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	
Genotype cohort (Meta-analysis of genotype distributions)												
Sanchez	2010	Spain	Case-control study	456, 85%	300,7%	108/348	75/225	51 ± 11	55 ± 4.5	141/199/109	99/150/46	0.382
Wang M	2017	China	Case-control study	620, NA	620, NA	105/515	108/512	28.28 ±8.92	27.83 ±7.58	178/272/167	216/278/124	0.046
Wang Q	2016	China	Case-control study	735, 100%	1204, NA	NA	472/732	NA		155/364/216	311/601/292	0.961
Bang	2010	Korea	Case-control study	1164, 96.5%	752, NA	140/1024	68/684	35.3 ± 9.8	31.4 ± 7.3	410/539/200	318/328/87	0.863
Wen	2014	China	Case-control study	475, 90.7%	527, NA	152/323	162/365	39.0 ± 11.3	39.0 ±11.9	101/218/82	112/216/141	0.103
Jung [a]	2016	Korea	Case-control study	285, 95.8%	363, 6.6%	31/254	77/286	23.4 ± 8.6	32.4 ± 10.3	63/163/59	181/151/31	0.950
Jung [b]	2016	Korea	Case-control study	576, 92.9%	680, 8.2%	82/494	102/578	28.7 ± 11.5	24.3 ± 14.7	173/323/79	290/298/90	0.333
Lin	2011	China	Case-control GWAS study	1837, NA	4231, NA	NA				221/438/273[d]	920/1584/786[f]	0.042
										179/446/280 [e]	252/450/238[g]	0.194
Provided OR-value analysis cohort (Meta-analysis of A vs. G)												
Lin				As above						A: G=1.26 (1.16–1.37)		As above
Bang				As above						A: G=1.33 (1.16-1.52)		As above
Wang M				As above						A: G=1.303 (1.111-1.526)		As above
Wang Q				As above						A: G=1.100 (1.034-1.171)		As above
Sanchez				As above						A: G=1.25 (1.01-1.54)		As above
Davidson	2014	China	Case-control GWAS study	775, NA	1587, NA	NA				A: G=1.16 (1.02-1.32) [h]		NA [c]
Reveille	2010	USA	Case-control GWAS study	2951, NA	6658, NA	NA				Discovery cohort: A: G=1.27 (1.18–1.37) Replication cohort: A: G=1.32 (1.18–1.49)		NA
Evans	2013	USA	Case-control GWAS study	WTCCC2: Case 1787, NA /Control 4800, NA TASC: Case 3023, NA /Control 8779, NA Replication study: Case 2111, NA /Control 4483, NA		NA				WTCCC2 Discovery cohort: A: G=1.32 (1.22–1.43) TASC Discovery cohort: A: G=1.34 (1.22–1.47) Replication analysis: A: G=1.36 (1.26-1.47)		NA
Zheng	2019	China	Case-control GWAS study	1860, NA	8883, NA	NA				A: G=1.26(1.17~1.35)		NA

a: Patients were in AS-GRS construction group in their study. b: Patients were in Independent validation set group in their study. c: *p*-value was 0.0005 or greater, but detail was not available. d: Genotyped by OmniExpress. e and f: Genotyped by 610quad. g: Genotyped by 1MDuo. h: Genotyping was performed in two round, meta-analysis of results of these two rounds was performed.

Table II. Results of rs10865331 polymorphism and AS susceptibility.

Genetic models	Number of studies	Test of association				Test of heterogeneity			Publication Bias				
		OR	95%CI	Z-value	*p*-value	Model	Chi-square	*p*-value	I^2	Begg	Egger	Number of potentially missing studies	Rosenthal's Fail-Safe Number
Recessive	9	1.376	1.204-1.572	4.68	<0.001	R	18.32	0.019	56.30%	<0.001	<0.001	0	158.84
Dominant		1.513	1.280-1.787	4.87	<0.001	R	35.55	<0.001	77.50%	<0.001	<0.001	0	310.35
Allele		1.336	1.214-1.470	5.92	<0.001	R	28.53	<0.001	72.00%	<0.001	<0.001	0	382.55
OR-value	12	1.268	1.213-1.325	10.55	<0.001	R	28.06	<0.001	60.08%	0.945	0.365	-	-
Pooled analysis	16	1.295	1.228-1.365	9.57	<0.001	R	57.13	<0.001	73.70%	0.558	0.074	-	-

The NOS assessments are shown in Supplementary file. The NOS assessments are shown in Supplementary file. Some clauses were added in some items to improve evaluation accuracy and the lowest score of all included studies was 6.

Statistical analysis

Statistical analysis was conducted in three cohorts: genotype analysis cohort, OR-value analysis cohort and pooled analysis cohort. The collected studies and data were applied in the subsequent statistical analysis. For some of the included studies that not met HWE, we deemed that these studies were unnecessary to be excluded and subgroup analysis were to be performed, and we observed that excluding these studies impacted little on the overall results. Meta-analysis results are shown in Table II.

In the genotype analysis cohort, a significantly positive correlation was detected in the three models. In each model, heterogeneity was greater than 50% and, therefore, a random model was used and the correlation estimation was relatively conservative. The summary OR value of the recessive model was 1.376 (95% CI: 1.204–1.572, $p<0.001$, $I^2=56.30\%$), which indicated a strongly positive correlation between rs10865331 and AS susceptibility. Similar outcomes were seen in the other two genetic models.

rs10865331 was positively correlated with AS susceptibility in view of the OR-value cohort. The summary OR value was 1.268 (95% CI: 1.213–1.325, $p<0.001$, $I^2=60.80\%$). In the pooled analysis cohort, the crude OR and 95% CI were calculated for the studies of Wen *et al*. (53), Lin *et al*. (29) and Jung's study (51), as well as our previous study (56); we merged the given OR and 95% CI with that of crude ORs in order for data in these studies to be included. The summary OR value was 1.295 (95% CI=1.228–1.365, $p<0.001$, $I^2=73.70\%$). In spite of the presence of heterogeneity, similar conclusions were still obtained. Forest plot of pooled analysis cohort is shown in Figure 2.

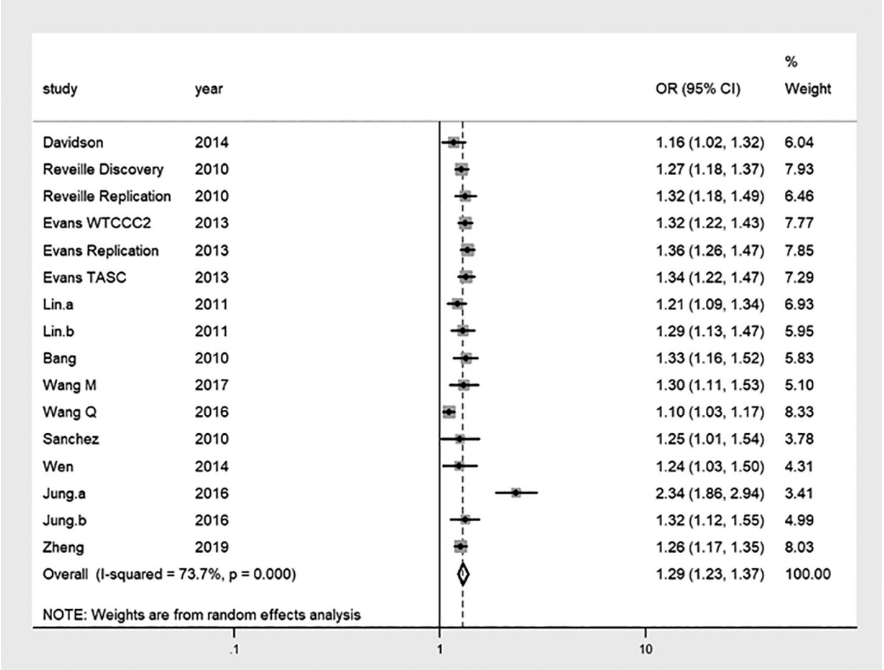

Fig. 2. Forest plot of pooled analysis.

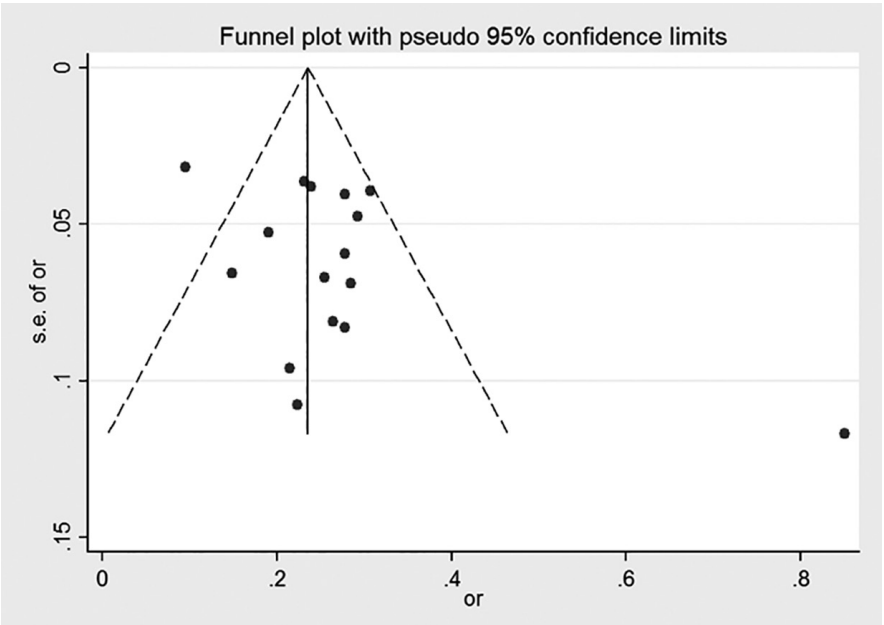

Fig. 3. Funnel plot of pooled analysis.

Subgroup analyses

Subgroup analyses were performed according to HWE, race, country, study type and source. In the genotype analysis cohort, only a Caucasian study was included, so only outcomes of the Asian subgroup were included in the race subgroup analysis. Overall, strong correlations were observed in most subgroups, and no significant difference was seen between subgroups. The results of subgroup analysis are shown in the Supplementary file.

Heterogeneity

In the genotype analysis cohort, heterogeneity was high and, thus, a sensitivity analysis was conducted. We observed that heterogeneity primarily stemmed from the scale construction set in the study by Jung *et al*. (51). We comprehensively reassessed this study to avoid bias, while causes of the large heterogeneity in the present study still remained unknown. After the sensitivity analysis, the heterogeneity of the recessive and dominant models could not be eliminated and we observed almost no change in the OR value and 95% CI.

In the OR-value and pooled analysis cohort, heterogeneity was high but could be eliminated. The study by Wang *et al*. (48) was detected as the source of heterogeneity, but we did not identify the reason why this study accounted for such large heterogeneity. Little impact was seen in the outcomes after this study was removed. The results and plot of sensitivity analysis are displayed in the Supplementary file.

Publication bias

Significant publication bias was obvious in all of the models in the genotype analysis cohort by Egger's linear regression test and Begg's rank correlation test, but not in the rest two cohorts, might due to sample size. To avoid omission, we re-conducted literature searching by terms "2p15 OR rs10865331" and no additional study was found. Thus, the Duval and Tweedie's nonparametric trim-and-fill analysis was performed and Rosenthal's fail-safe number was calculated. In the trim-and-fill analysis, we did not detect evidence of publication bias. Additionally, the fail-safe number of every model was much greater than 90 (5k+10, where k indicated the number of included studies; no more than 16 OR values and

95% CIs were included). These findings indicated that the outcomes of the meta-analysis were stable despite the existence of publication bias. A funnel plot of pooled analysis cohort is shown in Figure 3, and Rosenthal's fail-safe numbers are shown in Table II.

Discussion

Since rs10865331 was first detected as a biomarker of AS in 2010 (28), several relevant case-control studies have been conducted. The present study was designed to test the association between rs10865331, which is located in 2p15, and AS susceptibility. To the best of our knowledge, this HuGE review and meta-analysis provides the most comprehensive assessment of this correlation. In this study, we observed that rs10865331 strongly increased AS susceptibility in each race and country subgroup that we evaluated. The OR value in the pooled analysis was 1.295 (95% CI: 1.228–1.365, $p<0.001$, $I^2=73.70\%$), that was to say, this SNP brought about 30% more risk to AS. The stability of the outcomes was estimated by several methods and determined to be stable. This is the first systematic review and meta-analysis to confirm the relationship between rs10865331 and AS susceptibility.

Two intergenic regions, 2p15 and 21q22, were first detected as susceptibility areas in studies of Reveille *et al*. (28). The Australo-Anglo-American Spondyloarthritis Consortium (TASC) (28) subsequently validated the results. Further, 21q22 was confirmed as an AS risk region in a Zhuang population in China (57). To date, 2p15, the 23-kb region that includes SNPs such as rs10865331 and rs4672495 and plays a role in autoimmune functions, has not been extensively studied. However, several SNPs in this region have been identified as biomarkers of autoimmune disorders (58). Specifically, B3GNT2 was detected as a susceptibility locus of purely cutaneous psoriasis/psoriatic arthritis (59-62), systemic lupus erythematosus (63), Graves' disease, and rheumatoid arthritis (RA) (64-66). It functions in the biosynthesis of polyacetyl-lactosaminemine and its absence might result in hyperactivation of lymphocytes (67). Another SNP in this area, rs6759298 (68-69), was reportedly associated with the aetiology of AS and SpA with acute anterior uveitis (70-71), and, therefore, identified as an immune-related locus in a study of the International Genetics of Ankylosing Spondylitis Consortium (72). In a GWAS study, Reveille *et al*. (28) reported that long non-coding RNA transcripts were also identified by RNA-seq, indicating that these SNPs in 2p15 might exert impacts on non-coding RNA sequence or transcription effects.

Compared to SNPs not located in gene desert, few studies have examined the area of 2p15. It is located in an intergenic region and gene desert, so it is difficult to determine the associated gene, its function, and involved pathways. As an immune-related susceptibility locus, rs10865331 was identified as a risk locus in purely cutaneous psoriasis/psoriatic arthritis by a GWAS53, dense genotyping (73), and cellular dissection transcriptome analyses (74-76), but the same conclusion was not observed in psoriatic arthritis (73). In inflammatory bowel disease (IBD), rs10865331 was reported to be positively associated only with Crohn's disease but not with ulcerative colitis or general IBD (77-78); it was also not associated with RA (65). SpA and IBD are somehow associated, and rs10865331 has been commonly associated with both diseases (79): this SNP might be a target for explaining the mechanisms shared between these two diseases. Moreover, Jung *et al*. (51) developed a genetic risk scoring model for AS susceptibility prediction: it had positive applications in the study of AS risks, larger amount of, and more precise risk or prognosis models are, therefore, expected, especially in early identification of potential patients. Apart from these studies, we did not find any other studies examining this SNP. In addition, it was notable that rs10865331 and rs4672495 were in high linkage disequilibrium (28), but rs4672495 was not extensively studied. We only identified one study that reported that rs4672495 was able to influence B3GNT2 expression (65) and was associated with RA activity in a Chinese population in Taiwan, but its linkage disequilibrium was not further studied. Gene deserts are usually considered to be non-essential to genome function and, thus, neglected in terms of study. However, some gene deserts reportedly contain regulatory sequences that control the expression of distant genes (80) but, until now, little attention has been paid to the study of gene deserts.

In this systematic review and meta-analysis, we observed a strong correlation between rs10865331 and AS susceptibility, regardless of race or country of origin, indicating that this SNP has a universal impact on AS risk. High heterogeneity was detected in the majority of models and subgroups: sensitivity analyses were performed and more conservative outcomes were obtained. We also detected high publication bias, which we addressed in our analysis, and the data proved stable. Additionally, we performed literature searches twice to avoid omission. In general, this study confirmed the significance of rs10865331 in assessment of AS risk. However, in the study by Joshi *et al*. (81), rs10865331 was reportedly not associated with multiplex or singleton lesions in AS patients, which suggests that this SNP might affect only susceptibility and severity of AS (53). Little data has been published that describes the mechanism of how rs10865331 affects AS, the outcomes of treatment, or prognosis prediction.

Although the present study has many strengths, it also has several important limitations. First, numerous factors were involved in the pathogenesis of AS, and single SNPs have limited evidence to be a primary explanation for AS risks. Gene-environment and gene-gene interactions and the collaboration of multiple SNPs (82) in several genes can assess AS risk more accurately compared with single SNP. However, few relevant mechanism studies are available and it was impossible to further investigate this conclusion. Second, some of the provided OR values were not available in the OR-value cohort and the pooled analysis cohorts: we merged crude ORs with given/adjusted ORs in order to maximise the size of the sample. Although we

addressed the heterogeneity and publication bias, according to our previous study, heterogeneity and bias might still be a factor. In addition, some other factors, such as demographical, clinical indexes (83-84), may be also essential for AS pathogenesis. These factors are not all provided or adjusted in all included studies, although subgroup analyses are performed, it is still limited in estimating how much role these factors take. However, in spite of some limitations in meta-analysis concerning genes of AS, we make the most up-to-date and accurate estimates as possible (85).

In conclusion, the findings of the present HuGE review and meta-analysis suggest that rs10865331 increases AS susceptibility, regardless of race or country of origin. More precise studies are required to confirm and validate our findings. This study also highlights that further research is needed regarding AS pathogenesis, including the mechanisms of rs10865331 and gene deserts of 2p15.

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