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SYSTEMATIC REVIEW AND META-ANALYSIS

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The Association Between Genetic Polymorphism rs703842 in *CYP27B1* and Multiple Sclerosis

A Meta-Analysis

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Abstract: Multiple sclerosis (MS) is the most frequent nontraumatic disabling neurological disease among young adults. Previous studies have examined the association of rs703842 in *CYP27B1* with MS susceptibility, with inconsistent results reported.

The objective of this study is to conduct a systematic literature search and perform meta-analyses to examine whether rs703842 is associated with MS risk.

We searched potential literature in PubMed, Cochrane Library, Embase, Google Scholar, Web of Science, and HuGE by using the following inclusion criteria: studies were on human subjects; the studies were case–control studies; studies included subjects who had MS and those who did not have MS; and the studies provided genotype data for rs703842 for subjects who had and did not have MS, or provided odds ratios (ORs) and the 95% confidence intervals (CIs) for assessing the association of rs703842 with MS, or provided sufficient data for the calculation of OR and the 95% CI. We used random-effects models to calculate the OR as a measure of association. We used I^2 to assess between-study heterogeneity, and a funnel plot and Egger test to assess publication bias.

Seven studies published since 2008 met the eligibility criteria and were included in the meta-analyses. We found that the C allele was significantly associated with reduced MS susceptibility (OR = 0.88, 95% CI: 0.80–0.89; $P < 0.0001$). We also found significant association of rs703842 with MS risk using a dominant and a recessive model (both $P < 0.0002$). Our results remain unchanged if our meta-analysis was limited to studies that included only Caucasian participants (OR = 0.85, 95% CI: 0.80–0.90; $P < 0.0001$).

Our study has several limitations: The sample size is limited; We were unable to control for some important confounding factors as data for individual participant were not available; and Most of the included studies focus on MS risk in Caucasian. As a result, we could not perform meta-analysis for assessing the relationship in other ethnic groups.

In summary, we found that the genetic variant rs703842 in *CYP27B1* is associated with MS risk in Caucasians. More studies with larger sample size that control for important confounding factors are needed to validate the findings from this study.

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Abbreviations: CI = confidence interval, CYP27B1 = cytochrome P450 family 27 subfamily B member, EAE = experimental autoimmune encephalomyelitis, HWE = Hardy–Weinberg equilibrium, MS = multiple sclerosis, OR = odds ratio, SNP = single-nucleotide polymorphism.

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease in which local lymphocytic infiltration can damage myelin and axons, leading to formation of scar tissue, or sclerosis.[1] In the United States, it is estimated that there are about 400,000 people who are diagnosed with MS, with about 200 new diagnoses each week.[2] MS is the most frequent nontraumatic disabling neurological disease among young adults.[3] It causes enormous economic burden, such as treatment cost or loss in income due to inability or reduced ability to work. The annual treatment cost alone was estimated to be about $24,000 per patient in 2009.[4]

The exact etiology of MS remained unclear, and epidemiological studies indicate that it probably involves complex interaction between genetic and environmental factors.[5] A number of risk factors have been reported to be associated with MS susceptibility, such as gender,[6] ethnicity,[7] Epstein–Barr

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virus (EBV),[8] smoking,[9] latitude,[10] and vitamin D.[11] Notably, previous studies showed that MS incidence is inversely correlated to the degree of sunlight exposure.[12,13] The observation of higher MS prevalence in both hemispheres but decreased prevalence in the tropical areas led to the hypothesis that the sunlight effect on MS susceptibility may be through vitamin D production in the skin, which was confirmed from accumulating evidence.[14] Experimental autoimmune encephalomyelitis (EAE) is a model of MS. Previous research found that the development of EAE requires vitamin D,[15] and treatment of EAE mice with $1,25(OH)_2D_3$, the active form of vitamin D, or in combination with specific antigen myelin oligodendrocyte glycoprotein can potentially suppress or even block the development of EAE.[16] The exact mechanism linking vitamin D with MS etiology or MS development is still an active research area, and studies indicated that the positive effect of vitamin D on reducing MS risk might be attributed to its antiinflammatory influence.[17] Moreover, vitamin D can also promote recovery of central nervous system and can enhance neural stem cell proliferation and oligodendrocyte differentiation.[18]

Meanwhile, many genetic variants were found to be associated with MS risk. For example, HLA haplotypes, genetic loci at interleukin-2 receptor α (*IL2RA*), interleukin-7 receptor α (*IL7RA*), C-type lectindomain family 16 member A (*CLEC16A*), interferon regulatory factor 8 (*IRF8*), tumor-necrosis-factor receptor superfamily member 1A (*TNFRSF1A*), *CD6*, and *CD58*.[19–22] To date, previous GWAS have identified more than 100 loci that were associated with MS with genome-wide significance.[23] However, the association of most of these genetic variants is modest, with the exception of HLA-DRB1*15:01 haplotype which shows the strongest association with MS risk with an odds ratio (OR) of around 3.10.[23]

Cytochrome P450 family 27 subfamily B member (*CYP27B1*), located in 12q14.1, encodes a member of the cytochrome P450 superfamily of enzymes. *CYP27B1* plays a key role in converting vitamin D to its active form, 1,25-dihydroxyvitamin D_3, and therefore, is essential in regulating the level of biologically active vitamin D and calcium homeostasis.[24] Given the possible link of vitamin D with MS susceptibility, it is anticipated that genetic variants in *CYP27B1* might have an influence on MS risk. Indeed, previous studies have reported the association of MS with multiple genetic variants in *CYP27B1*, such as rs118204009,[25] rs12368653,[26] and rs10876994.[26] Many studies also examined the association of the single-nucleotide polymorphism (SNP) rs703842 in *CYP27B1* with the risk of MS, with inconsistent results reported.[27–33] In this study, we conducted a systematic literature search and performed meta-analyses to investigate the association between rs703842 and MS susceptibility.

METHODS

Eligibility Criteria

The following criteria were used for assessing study eligibility: studies were on human subjects; the studies were case–control studies; studies included subjects who had MS and those who did not have MS; and the studies provided genotype data for rs703842 for subjects who had and did not have MS, or provided ORs and the 95% confidence intervals (CIs) for assessing the association of rs703842 with MS risk, or provided sufficient data for the calculation of OR and the 95% CI. Studies were excluded if: they were unpublished; they were abstracts/comments, reviews, or meta-analyses; and there were no control group. If overlapping data were used, we chose the study with a larger sample size.

Search Strategy

Two authors (LL and JY) performed an independent and extensive literature search in PubMed, Cochrane Library, Embase, Google Scholar, Web of Science and HuGE (a navigator for human genome epidemiology) for papers published before October 13, 2015. The keywords used in the literature search can be found in the online supplementary file, http://links.lww.com/MD/A948.

We retrieved all potentially relevant studies to evaluate their eligibility, and also hand searched the references in all included studies for possible studies that were missed in the literature search. The search was limited to studies published in English. No efforts were made to contact the authors for additional data. A group discussion was held to resolve any disagreement until a consensus was reached.

Data Extraction

Two authors (YB and JY) extracted the following data from the eligible studies: name of the first author, year of publication, mean age, distribution of gender, ethnicity of the participants, genetic models used for analysis, rs703842 genotype data for patients with and without MS, or OR and the corresponding 95% CI. The quality of the included studies were assessed independently by 2 authors (TJ and JY) using Newcastle–Ottawa scale.[34]

Data Analysis

We used ORs to assess the association between rs703842 and MS susceptibility. In all meta-analyses, the ORs were calculated using random-effects models. We used I^2 to assess between-study heterogeneity, and a funnel plot and Egger test to assess publication bias.

If a study reported adjusted OR and the corresponding 95% CI for a specific genetic model, we used that information for the meta-analysis for that genetic model, even though crude OR or genotype data to calculate the crude OR were available.

As a systematic review and meta-analysis, ethical approval of this study is not needed. This work was reported according to the PRISMA guidelines.[35] All statistical analyses were performed using Stata 11.2 (StataCorp LP, College Station, TX). A P-value <0.05 was considered statistically significant.

RESULTS

Study Selection and Characteristics

Literature search and selection of eligible studies are shown in Figure 1. In our initial search, we identified a total of 114 potential publications. Among them, 93 publications were excluded because they were irrelevant, reviews/abstracts, not about human subjects, or not published in English. We retrieved the remaining 21 papers for a more detailed evaluation and further excluded 14 studies because they were not case–control studies, there were insufficient data or they were not about rs703842, leading to 7 relevant publications to be included in our analyses.[27–33]

All the included studies were published since 2008. The sample size ranged from 150 to 8,004. The meta-analysis included a total of 22,851 participants. All included studies examined the association of rs703842 with MS in Caucasians

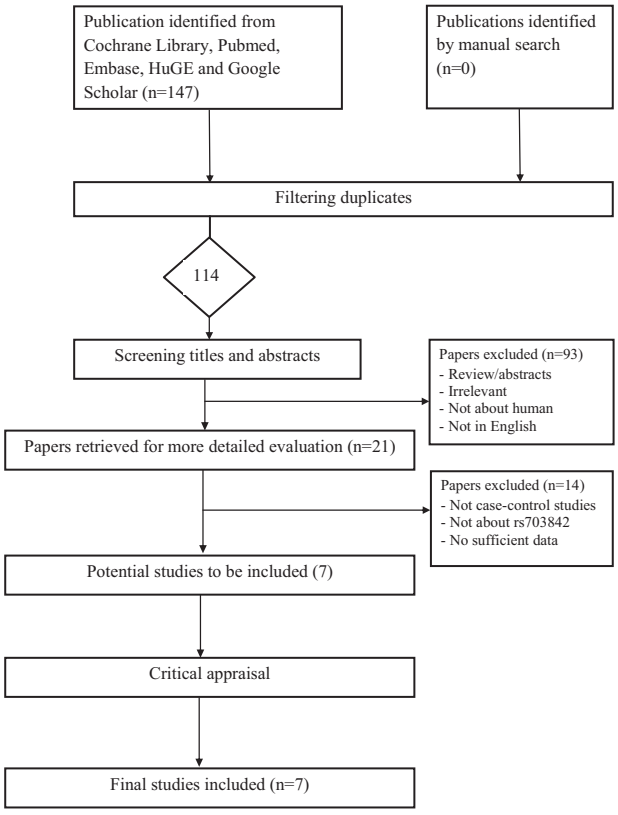

FIGURE 1. Flow diagram of the selection process of the studies included in the meta-analyses. Note: Please see the Methods section for additional details.

except one,[27] which examined the association in Chinese (Table 1).

Assessment of Publication Bias

We found no evidence of publication bias for the meta-analysis of rs703842 with MS using the allelic model ($P = 0.49$; Figure 2). Assessment of publication bias for the meta-analyses using other genetic models is not meaningful due to limited number of studies included in the corresponding analyses.

Association of rs703842 With MS

Of the 7 studies included in our meta-analysis, only 3 studies provided genotype data for participants with and without MS.[27,30,32] The other 4 studies provided adjusted OR and the corresponding 95% CI for the association of rs703842 with MS.[28,29,31,33] Therefore, meta-analysis using allelic models utilized results from all the 7 studies, while meta-analysis using other genetic models (dominant, recessive, and additive) is only available in the 3 studies that reported genotype data.

All the studies seemed to indicate that the C allele in rs703842 was associated with decreased risk of MS, although the association was statistically significant in only 3 studies (Figure 3).[28,30,31] Our meta-analysis showed that the C allele in rs703842 was significantly associated with reduced MS susceptibility (OR = 0.85, 95% CI: 0.80–0.89; $P < 0.0001$). There was no significant heterogeneity between studies ($I^2 = 14.9\%$, $P = 0.316$).

We found a significant association between rs7013842 and MS risk using a dominant model (OR = 0.75, 95% CI: 0.63–0.89: $P = 0.001$) and a recessive model (OR = 0.85, 95% CI: 0.77–0.94; $P = 0.002$), but no significant association was found using an additive model (OR = 0.87, 95% CI: 0.71–1.06; $P = 0.164$). However, this result should be interpreted with caution because only 3 studies were included in the meta-analysis.[27,30,32]

Sensitivity Analysis

We repeated our analysis by excluding studies that did not meet Hardy–Weinberg equilibrium (HWE) or those for which HWE information could not be obtained.[28,30,33] The C allele in rs703842 remained to be associated with MS risk (OR = 0.88, 95% CI: 0.82–0.95; $P = 0.001$). There was no significant heterogeneity between studies ($I^2 = 0\%$, $P = 0.795$).

The observed association between the C allele in rs703842 and MS risk remained if our meta-analysis was limited to studies that included only Caucasian participants (OR = 0.85, 95%CI: 0.80–0.90; $P < 0.0001$). There was no significant heterogeneity between studies ($I^2 = 24.8\%$, $P = 0.248$). The

TABLE 1. Basic Characteristics of the Studies Included in the Meta-Analyses

Study (Author, year) [Reference]	Race/Country	MS			Control			NOS
		N	Age (Mean ± SD)	Male (%)	n	Age (Mean ± SD)	Male (%)	
Zhuang et al, 2015[27]	Asian	116	NA	NA	301	NA	NA	6
Cortes et al, 2013[28]	Caucasian	3269	NA	NA	3577	NA	NA	6
Orton et al, 2011[29]	Caucasian	1364	NA	378 (27.8%)	1661	NA	802 (48.3%)	6
Simon et al, 2011[30]	Caucasian	1655			6349			6
Sundqvist et al, 2010[31]	Caucasian	2158	NA	612 (28.4%)	1759	NA	585 (33.3%)	5
Simon et al, 2010[32]	Caucasian	214	NA	0	428	NA	0	9
Orton et al, 2008[*,33]	Caucasian	NA	NA	NA	NA	NA	NA	9

NOS is a validated tool for assessing the quality of nonrandomized studies in meta-analyses. CI = confidence interval, MS = multiple sclerosis, NA = not available, NOS = the Newcastle–Ottawa Scale, OR = odds ratio, SD = standard deviation.

[*]The paper only indicated that a total of 150 twin subjects were included for genotyping (adjusted OR and 95% CI were used). Such studies can be included in our studies because if a study reported adjusted OR and the corresponding 95% CI for a specific genetic model, we used that information for the meta-analysis for that genetic model, even though crude OR or genotype data to calculate the crude OR were available.

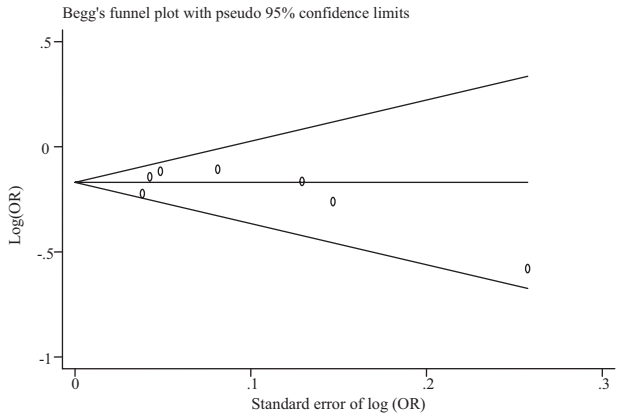

FIGURE 2. Funnel plot for meta-analysis of the association of rs703842 with multiple sclerosis. The x-axis is the standard error of the log-transformed OR (log [OR]), and the y-axis is the log-transformed OR. The horizontal line in the figure represents the overall estimated log-transformed OR. The 2 diagonal lines represent the pseudo 95% confidence limits of the effect estimate. OR = odds ratio.

observed association remained unchanged if we excluded the study that showed the most significant association[28] (OR = 0.87, 95% CI: 0.82–0.92; $P < 0.0001$). There was no significant heterogeneity between studies ($I^2 = 0\%$, $P = 0.545$). Excluding studies of low quality[31] did not change our findings (OR = 0.83, 95% CI: 0.79–0.88; $P < 0.0001$). There was no significant heterogeneity between studies ($I^2 = 9.5\%$, $P = 0.355$). Finally, to minimize the influence of a possible, albeit very unlikely, overlapping of data, we reran the analysis by excluding 1 earlier study,[33] and our results remain unchanged (OR = 0.85, 95% CI: 0.81–0.89; $P < 0.0001$). There was no significant heterogeneity between the studies ($I^2 = 0\%$, $P = 0.481$).

DISCUSSION

In this study, we conducted a systematic literature search and performed meta-analyses to assess the association of rs703842 in *CYP27B1* with MS. We found that the C allele showed significant association with reduced MS risk in Caucasians. The association of rs703842 with MS susceptibility did not change under different genetic models, except the additive model, probably because of reduced power due to a limited number of the included studies. The association remained when we excluded studies that violate HWE. To the best of our knowledge, this is the 1st meta-analysis on the association of rs703842 with MS susceptibility.

Previous studies have identified multiple genetic, epigenetic, and environmental risk factors that are associated with MS susceptibility.[36–38] Notably, high MS frequency occurs in areas with low sunlight exposure, a major inducer of previtamin D synthesis in the skin.[12] A battery of epidemiologic, experimental, and clinical evidence also suggests a link between hypovitaminosis D and increased MS susceptibility and relapses.[11,17,39,40] *CYP27B1* encodes the enzyme 25-hydroxyvitamin D-1 alpha hydroxylase, which hydroxylates 25-hydroxyvitamin D into the bioactive form $1,25(OH)_2$ vitamin D. This active metabolite is a potent immuno-modulator important for

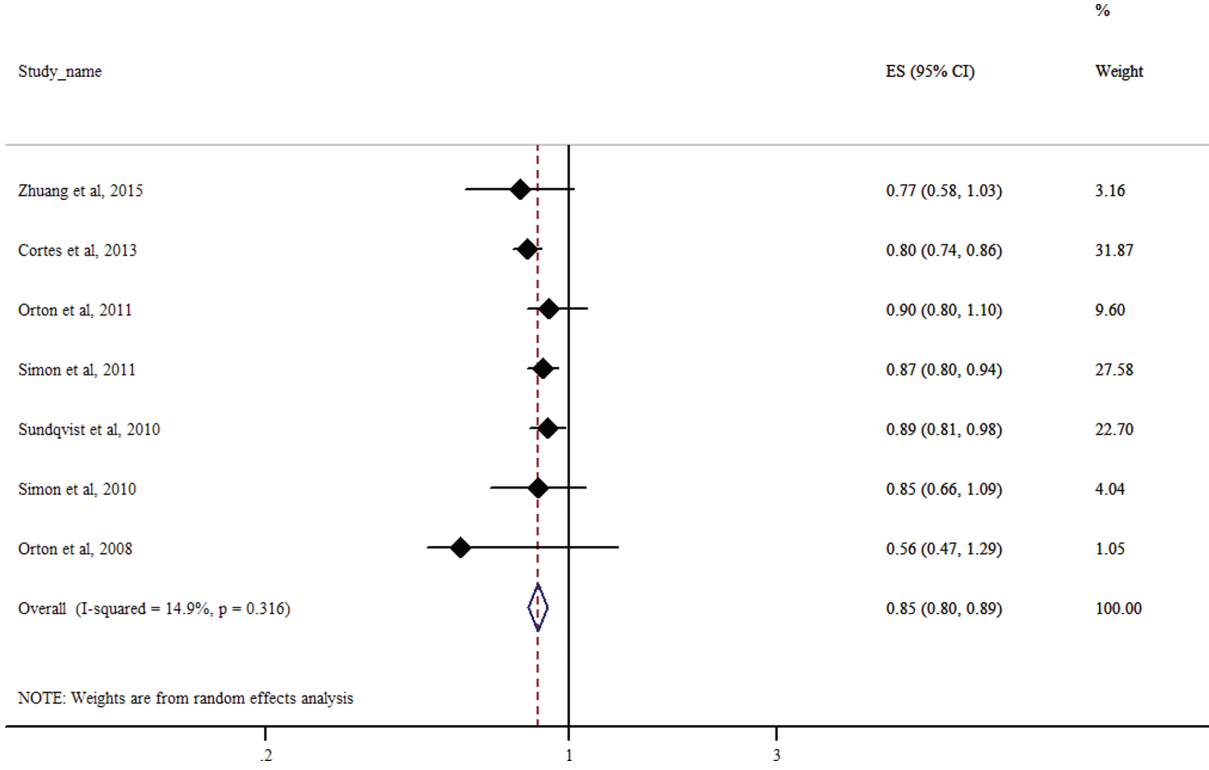

FIGURE 3. Forest plot for meta-analysis of the association of rs703842 with multiple sclerosis using the allelic model (C vs T). Each study is represented by a square whose area is proportional to the weight of the study. The overall effect from meta-analysis is represented by a diamond whose width represents the 95% CI for the estimated OR. CI = confidence interval, OR = odds ratio.

immune function and development, including innate and adaptive immunity, immune tolerance and B-cell homeostasis, and type 2 antiinflammatory T helper cell generation.[41–44] Recent studies have examined the association of MS with multiple genetic variants in vitamin D metabolizing genes, such as p.R389H mutation, rs12368653, rs10876994, rs118204009 and rs703842 in *CYP27B1*, and rs2248359 in *CYP24A1*.[25,28,45] A previous meta-analysis found no association of 2 common SNPs (rs2228570 and rs731236) in the vitamin D3 receptor gene (*VDR*) with risk for MS.[46] Another more recent meta-analysis of 4 polymorphisms in *VDR* (rs2228570, rs731236, rs1544410, and rs7975232) confirmed no association of rs731236 with MS risk, but found a significant association of rs2228570 using a dominant and codominant model.[47] The conflicting results from these studies are probably due to different study designs, different genetic models used, or the heterogeneity of the ethnic background of the study participants.[25,48,49] Further studies are required to clarify the relationship between these genetic variants and MS risk.

The SNP rs703842 lies 1.76 kb upstream of *CYP27B1* and in the 3′-untranslated region of the neighbor gene methyltransferase-like protein 1 (METTL1). Whether and how this genetic variant regulates CYP27B1 expression and vitamin D metabolism remains unclear. A previous twin study identified 2 SNPs in *CYP27B1* (rs703842 and rs4646536) as significant predictors of 25(OH)D concentrations.[33] This finding, however, could not be replicated in other studies.[50,51] Another study found that rs703842 was associated with altered expression level of a proximal gene Ts translation elongation factor, mitochondrial, and another strong MS candidate gene.[52] Interestingly, this chromosomal region harboring *METTL1-CYP27B1-CDK4* genes was also found to be associated with some other autoimmune diseases, such as type 1 diabetes, coeliac disease, and rheumatoid arthritis.[53–57] More studies are needed to clarify the functional role of rs703842.[58]

Since previous studies suggested that lower level of blood vitamin D concentration is an important risk factor that can influence MS susceptibility, in clinical practice, it might be valuable to assess the level of blood vitamin D as well as to genotype variants associated with MS risk, such as rs703842.[59] Previous studies reported beneficial effects of vitamin D supplementation in MS patients.[60,61] Therefore, oral supplementation could be an alternative way to improve vitamin D level other than sunlight exposure for the prevention and treatment of MS. Preclinical studies and RCTs focusing on the safety and efficacy of vitamin D supplementation are undergoing.[62]

Our study has some limitations: The sample size is still limited despite our efforts to perform a literature search as systematic as possible; Because only published data were used, we were unable to control for some important confounding factors such as age, gender, and smoking as data for individual participant were not available; and Most of the included studies focus on MS risk in Caucasian. As a result, we could not perform meta-analysis for assessing the relationship in other ethnic groups, and our results might not be generalized to other ethnicities.

In summary, in this study, we conducted meta-analyses to evaluate the association between rs703842 in *CYP27B1* and MS susceptibility. We found that the C allele was associated with lowered MS risk in Caucasians. Whether the association holds for other ethnic groups needs further investigation. More studies with larger sample size that control for important confounding factors are also needed to validate the findings from this study.

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