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META-ANALYSIS

No Evidence for an Association Between the Chemokine Receptor 5 Δ32 Polymorphism and Multiple Sclerosis Susceptibility: A Meta-Analysis

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Abstract

Background/Aim: Numerous reports have been published on the association of the chemokine receptor 5 Δ32 genetic variation (rs333) with the risk of multiple sclerosis (MS), with results that are inconsistent. The relationship between rs333 and susceptibility to MS was evaluated in this study.
Methods: The PRISMA guidelines were followed in the current study. Twelve databases were used to find eligible articles. The investigators extracted the necessary information. The associations of the alleles and genotypes were evaluated in different models of inheritance: co-dominant, dominant and recessive genotype models and allele model.
Results: The analysis included 14 articles reporting 16 studies involving 3265 MS patients and 3735 healthy controls. There was no substantial heterogeneity between studies for any of the comparisons. The significance level was not reached for the association between rs333 and MS susceptibility.
Conclusion: The findings of this study could not confirm the relationship between the rs333 and susceptibility to multiple sclerosis.

Key words: CCR5; Receptors, CCR5; Δ32; Multiple sclerosis; Meta-analysis.

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Introduction

Multiple sclerosis (MS) is one of the best studied autoimmune diseases. It is generally accepted that central nervous system inflammation in MS is hallmarked by demyelination and damage to axons, leading to progressive neuronal dysfunction. The cause of MS is not well understood. However, genetic studies suggest it has heritability.[1-4]

Chemokine receptors show an essential role in inflammation by controlling immune cell migration. The human C-C chemokine receptor 5 gene (*CCR5*, MIM: 601373) encodes a chemokine receptor found on the surface of leukocytes.[5] *CCR5* is also known as *CCCKR5*, *CMKBR5*, *CKR5*. The human *CCR5* gene has several genetic polymorphisms, of which a deletion of 32 bp (Δ32, rs333) is the most studied ones. This polymorphism results in a frameshift and premature translation termination of the protein.[5, 6] The result of heterozygosity for the Δ32 allele is a reduction of the receptor and the result of homozygosity for this allele is the absence of an active receptor. Studies have shown that ethnic groups have very different patterns of Δ32 allele frequency in their gene pools, with the highest frequency in Caucasians and very low frequency in East Asian and African ethnic groups.[7, 8]

There are too many published studies that have evaluated the relationship between rs333 and a very wide range of multifactorial diseases; we have tried to mention a few of these studies. The non-functional receptor produced by the Δ32 allele is resistant to infection of human immunodeficiency virus-1.[6,9] Based on ecological studies, the Δ32 allele is highly associated with the outcome of COVID-19.[10,11] A study suggests that the variant allele is negatively associated with COVID-19.[12]

There have been studies examining the relationship between rs333 and the risk of autoimmune disease, with inconsistent results.[13-17] Several studies have examined the association between rs333 and susceptibility to MS,[18-36] but the results have been inconsistent. Therefore, making it difficult to understand the true association of this polymorphism with MS. To cover the weakness of studies with small number of participants and to increase the statistical power, meta-analysis of published data is recommended.

Meta-analysis is a very useful and popular statistical method to integrate and compare the data of several reports and to overcome the weakness of single genetic association studies, especially when previous studies have failed to show significant associations due to small sample size and/or low statistical power. In the middle of the last decade, a meta-analysis[37] was published using 8 original articles. It should be noted that this meta-analysis suffers from the authors' imprecise search for relevant articles.

Aim of this study was to examine the relationship between the rs333 and the risk of multiple sclerosis.

Methods

Search strategy

The current study was conducted according to the PRISMA guidelines. Eligible articles were identified by systematic searches of 12 databases, including *PubMed*, *Scopus*, *Europe PMC*, *DOAJ*, *EBSCO*, *ISC*, *AJOL*, *Cochran Library*, *EMBASE*, *Scilit*, *SCIndex* and *KoreaMed*. The search was performed on 31 August 2024. The keywords used were "multiple sclerosis" AND *CCR5* AND polymorphism. By reviewing the references of eligible articles, other relevant studies were recognised.

Study criteria

The criteria for inclusion of an article in this study were as follows: case-control design; raw genotypic data available; studies written in English. Published case reports, reviews, meta-analyses, case-only reports, letters, abstracts presented at conferences, articles reporting duplicate data and non-English articles were excluded. Articles reporting linkage analysis were also excluded. Finally, studies in which there was a statistically significant difference between the observed and HWE-expected genotype frequencies in their healthy controls were excluded because, as noted elsewhere,[38-40] genotyping error or sampling bias may be involved in such a situation.

Data extraction

The following data was extracted: author name, country of study, publication date, number of MS patients and controls, ethnicity of participants, method used to determine genotype, method used to select control group. The following information was extracted separately for MS cases and controls: mean age of participants, percentage of female participants and genotype frequencies of the rs333 polymorphism. In the articles where participants belonged to different ethnic groups and genotyping data were available for ethnic groups, the data were extracted separately and each data set was considered as one study. The two sets of data extracted by the researchers were similar without any discrepancies.

Assessment of the quality score

The quality of the studies included in the meta-analysis was assessed on the basis of both traditional epidemiological considerations and association genetic issues as previously used,[41] with minor modifications (Table S1). The predefined scales used were source of cases, source of controls, statistical analysis, Hardy-Weinberg equilibrium in controls and total sample size. The quality of the studies was assessed independently by the authors. Disagreements were resolved by discussion between the two reviewers. Overall scores ranged from 0 (worst) to 15 (best). Reports with a score of less than 10 were classified as "low quality" and those with a score of 10 or more were classified as "high quality".

Statistical analysis

The association strength was reported using odds ratio (OR). A 95 % confidence interval (CI) was also computed for the OR. An OR less than or greater

than 1 was considered a protective or risk factor, respectively. I^2 statistics and Cochran's Q test were used for evaluated heterogeneity between studies. Q statistic with $p < 0.10$ considered significant, indicating that the studies had significant heterogeneity. I^2 values of less than 25 %, between 26 and 50 %, 51 and 75 % and greater than 0.75 % show low, moderate and high heterogeneity, respectively. In the presence and absence of heterogeneity, the random effects model and the fixed effects model were used, respectively.[42, 43]

The relationship between polymorphism and risk of MS has been studied in different models of inheritance. The stability of the associations studied was assessed by performing a sensitivity analysis. The possibility of publication bias and its effect on the meta-analysis results were assessed using Egger's regression test and funnel plot.[44] The Comparative Meta-Analysis software (ver. 2.2.064, USA) was used.

Results

The search strategy and data sources are shown in the PRISMA flowchart (Figure 1).

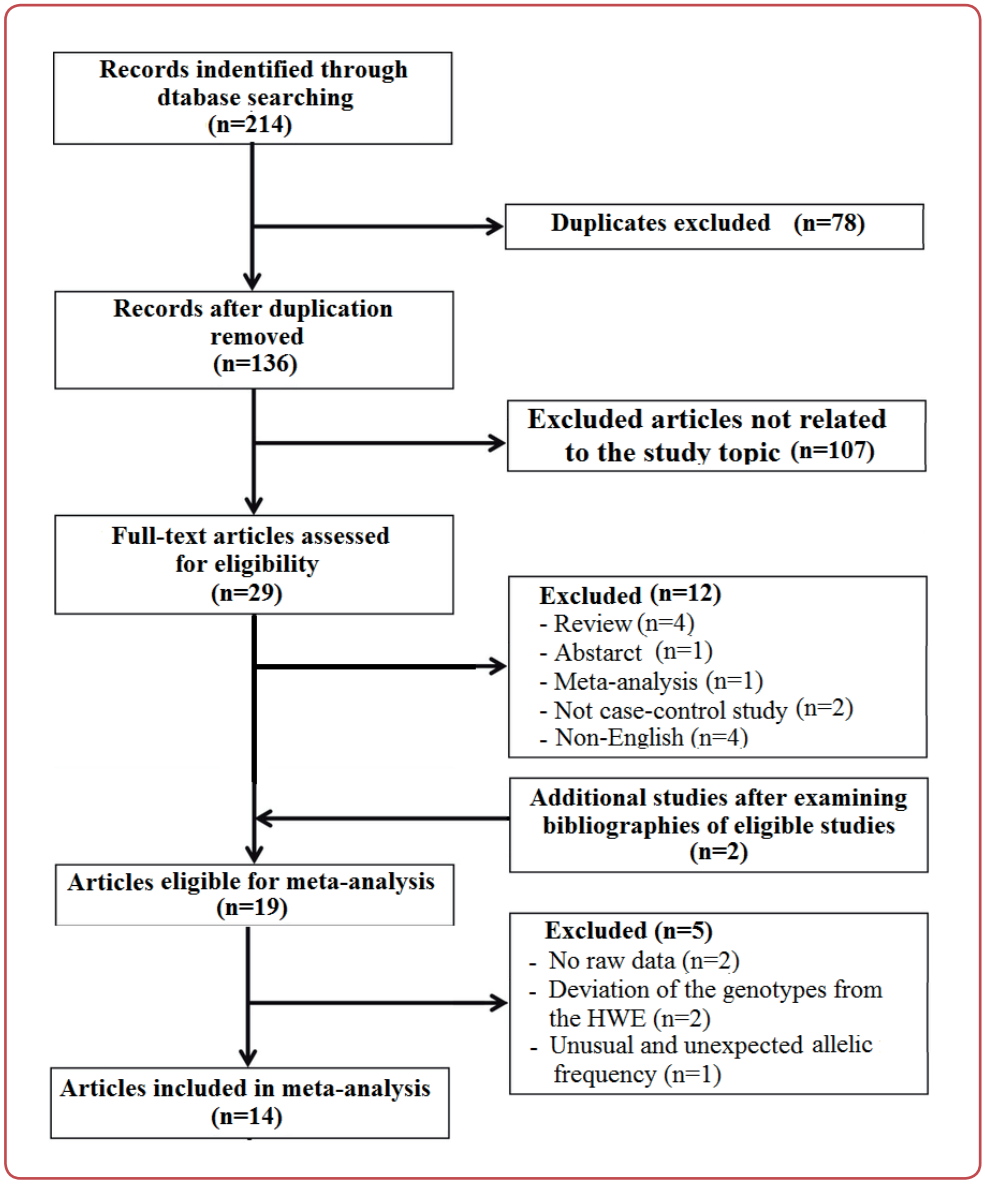

Figure 1: Flow diagram for identifying and including studies in the current meta-analysis

Twelve databases were searched, identifying 214 articles. Duplicate articles were removed from the list (n = 78) and another 107 articles were removed because they were unrelated to the study topic. During the search process, the full text of 29 articles was assessed to identify eligible articles. Finally, 19 eligible articles were reviewed. Of these, two studies by D'Angelo and colleagues[32] and Gade-Andavolu and colleagues[22] were excluded because they did not report raw genotypic (or allelic) data. The observed and expected genotypic values of healthy controls in two other studies[26, 30] showed significant differences. As mentioned elsewhere,[41-43] genotyping error or sampling bias may be involved in such a situation. Therefore, these two studies were also excluded. Kazemi-Arababadi et al[31] reported a study with 100 cases and 300 controls from Iran. They reported that the Δ32 allele was absent in their MS cases and the variant allele was present in only 2 heterozygous controls, giving an allele frequency of about 0.0033 in controls, which is a very unusual and unexpected allele frequency when compared with its worldwide frequency[7,8] and also other reports from the Iranian gene pool.[36, 45, 46] This allele frequency is very similar to that of the East Asian population. This very large difference indicates that some errors occurred during genotyping, so this article was also excluded. After excluding these five studies, the final number of articles for analysis was 14. The reports by Otagui et al[29] and Troncoso et al[35] included participants from two ethnic groups. Therefore, each of these articles was considered as two studies. Therefore, a total of 16 studies with a total of 3,265 MS patients and 3,735 healthy volunteers were included in the meta-analysis (Table 1).

The publication dates ranged from 1997 to 2021 and all had a case-control design. There were 14 and 2 studies conducted in Caucasian and African populations, respectively. In all studies, genotypes were determined by PCR. The number of participants (controls plus patients) ranged from 190 to 1256. The quality scores of the individual studies ranged from 7 to 13, with 75.0 % (12 out of 16) of the studies classified as high quality.

Table 2 shows the genotype frequencies in the MS cases and controls of the studies. As mentioned in the methods section, the associations under four genetic models, was evaluated.

Table 1: Characteristics of the original articles included in the meta-analysis

First author	Year	Country	Ethnicity	Source of controls	Cases			Controls			Quality score
					N	Age	Female proportion	N	Age	Female proportion	
Bennetts	1997	Australia	Caucasian	-	120	-	-	168	-	-	7
Sellebjerg	2000	Denmark	Caucasian	BD, HSM	148	Median 36	73 %	151	-	-	8
Favorova	2002	Russian	Caucasian	-	219	-	-	354	-	-	8
Pulkkinen	2004	Finland	Caucasian	HSM	89	46	-	111	45.7	-	10
Luomala	2003	Finland	Caucasian	Medical staff and tampere residents	116	46	57.7 %	109	46	-	11
Silversides	2004	North Ireland	Caucasian	BD	439	-	-	230	-	-	11
Ristic	2004	Slovenia and Croatia	Caucasian	BD	325	-	71 %	356	-	-	11
Sellebjerg	2007	Denmark	Caucasian	BD, HSM	109	Median 42	75 %	105	Median 38	70 %	10
van Veen	2007	Netherlands	Caucasian	Students and staff of university	637	49	66 %	177	43	54 %	12
Otagui-1	2007	Spain	Caucasian	BD	62	-	-	139	-	-	10
Otagui-2	2007	Spain	Caucasian	BD	102	-	-	210	-	-	10
Torok	2015	Hungary	Caucasian	-	428	43.7	75 %	828	44.3	75 %	13
Karam	2016	Egypt	African	-	80	35.5	67 %	110	34.8	60 %	10
Troncoso-1	2018	Brazil	Caucasian	-	166	48.5	-	235	-	-	11
Troncoso-2	2018	Brazil	African	-	74	47.9	-	200	-	-	11
Asgharzadeh	2021	Iran	Caucasian	-	152	32.1	-	242	31	-	7

BD: blood donors; HSM: Hospital staff members; PB: population-based control; N: number of cases;

Table 2: Chemokine receptor 5 delta32 (CCR5-Δ32) genotypes in multiple sclerosis patients and controls of studies used in the meta-analysis

First author	Year	Ethnicity	Cases			Controls			p-value for HWE
			wt/wt	wt /Δ32	Δ32/ Δ32	wt/wt	wt /Δ32	Δ32/ Δ32	
Bennetts	1997	Caucasian	95	23	2	137	30	1	0.991
Sellebjerg	2000	Caucasian	115	32	1	110	39	2	0.479
Favorova	2002	Caucasian	166	49	4	286	66	2	0.383
Luomala	2003	Caucasian	92	17	7	84	26	1	0.509
Pulkkinen	2004	Caucasian	71	12	6	88	30	1	0.364
Silversides	2004	Caucasian	331	100	8	165	58	7	0.495
Ristic	2004	Caucasian	285	37	3	312	41	3	0.212
Sellebjerg	2007	Caucasian	87	21	1	80	23	2	0.817
van Veen	2007	Caucasian	515	112	9	145	31	1	0.632
Otagui-1	2007	Caucasian	60	2	0	116	22	1	0.969
Otagui-2	2007	Caucasian	88	12	2	172	37	1	0.506
Torok	2015	Caucasian	352	71	5	670	146	12	0.216
Karam	2016	African	50	23	7	70	35	5	0.815
Troncoso-1	2018	Caucasian	155	11	0	202	31	2	0.509
Troncoso-2	2018	African	71	3	0	194	6	0	0.829
Asgharzadeh	2021	Caucasian	144	8	0	224	18	0	0.547

1. Co-dominant model: The results of the analysis under this assumption are shown in Figure 2. Statistical analysis showed that homozygosity was not associated with the risk of MS (OR = 1.28, 95 % CI: 0.81-2.03, p = 0.276). The wt/Δ32 genotype was weakly associated with the risk of MS (OR = 0.87, 95 % CI: 0.75-0.99, p = 0.044). The heterogeneity between studies was not significant in either analysis (for homozygosity: Q = 14.01, df = 13, p = 0.373; I^2 = 7.23 %; for heterozygosity: Q = 17.31, df = 15, p = 0.300; I^2 = 13.38 %).

2. Dominant model: The results showed that the comparison was not significant (Figure 3) (OR = 0.90, 95 % CI: 0.79-1.02, p = 0.121) and the heterogeneity between studies was not significant (Q = 16.75, df = 15, p = 0.334; I^2 = 10.47 %).

3. Recessive model: The analysis showed that the recessive genotype was not associated with the risk of MS (Figure 4) (OR = 1.32, 95 % CI: 0.84-2.09, p = 0.219) with no significant heterogeneity (Q = 14.23, df = 13, p = 0.357; I^2 = 8.68 %). Note that two studies[35, 36] were not included in the analysis because they did not observe the recessive genotype in their participants.

4. Allelic model: There was no heterogeneity (Figure 5) (Q = 19.03, df = 15, p = 0.212; I^2 = 21.21 %) and no relationship between the Δ32 allele and MS risk (OR = 0.94, 95 % CI: 0.84-1.06, p = 0.365).

Sensitivity analysis was performed to evaluate the influence of each study on the strength of association and it showed that almost none of the studies remarkably affected the associations, except for the comparison of Δ32/Δ32 vs wt/wt. This means that the present results were robust. It should be noted that in the comparison of Δ32/Δ32 vs wt/wt, the removal of several studies from the analysis affected the OR and the significance level. This clearly shows that the observed relationship was not only weak, but also highly dependent on some studies and cannot be trusted.

Typically, in the presence of heterogeneity, researchers have often attempted to identify its source(s), usually by stratifying data based on source of controls, genotyping method, sample size, etc. Here, because the ethnicity of the majority of participants and the genotyping method were the same and no heterogeneity was found between studies, no further analysis was considered necessary.

Publication bias is a major problem for meta-analyses because of the disproportionate number of studies with positive results. Finally, publication bias was not detected.

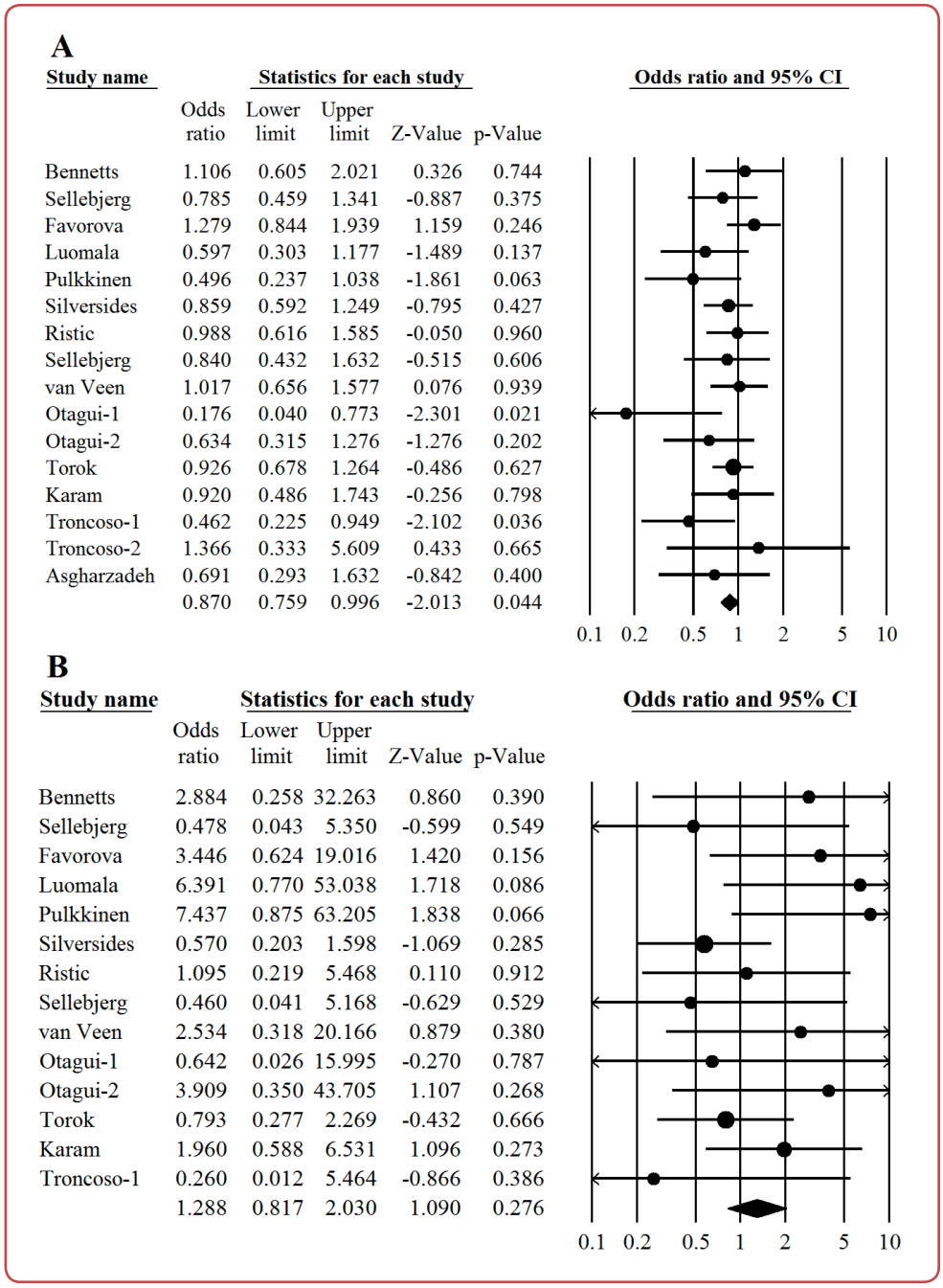

Figure 2: Forest plot of the association between chemokine receptor 5 delta32 (CCR5-Δ32) polymorphism and multiple sclerosis risk: A) wt/Δ32 vs wt/wt. B) Δ32/Δ32 vs wt/wt

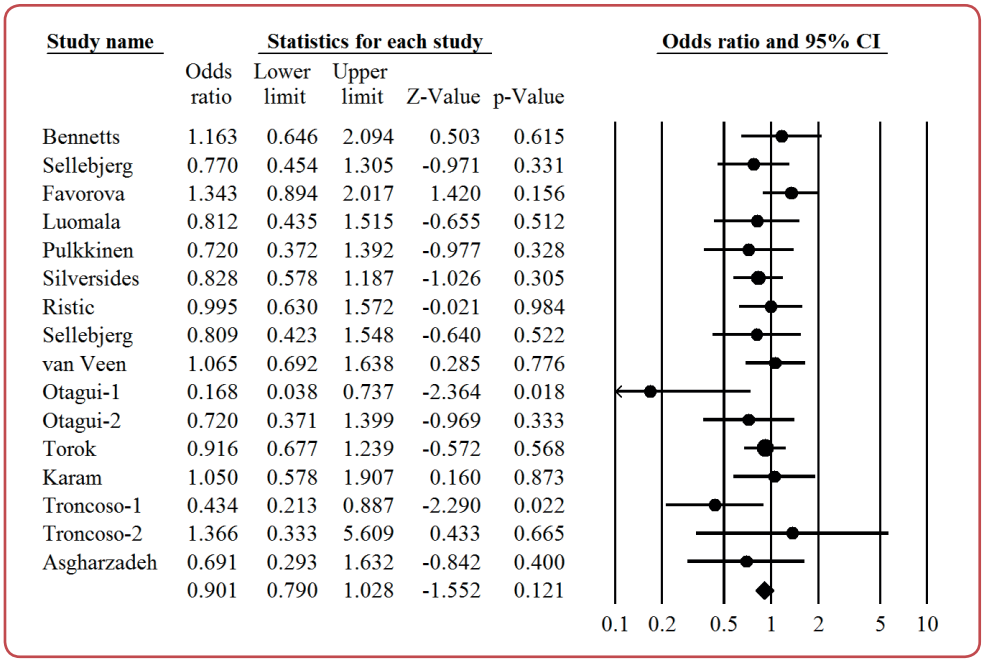

Figure 3: Forest plot of the association between chemokine receptor 5 delta32 (CCR5-Δ32) polymorphism and multiple sclerosis risk. Assuming the Δ32 allele act as a dominant allele

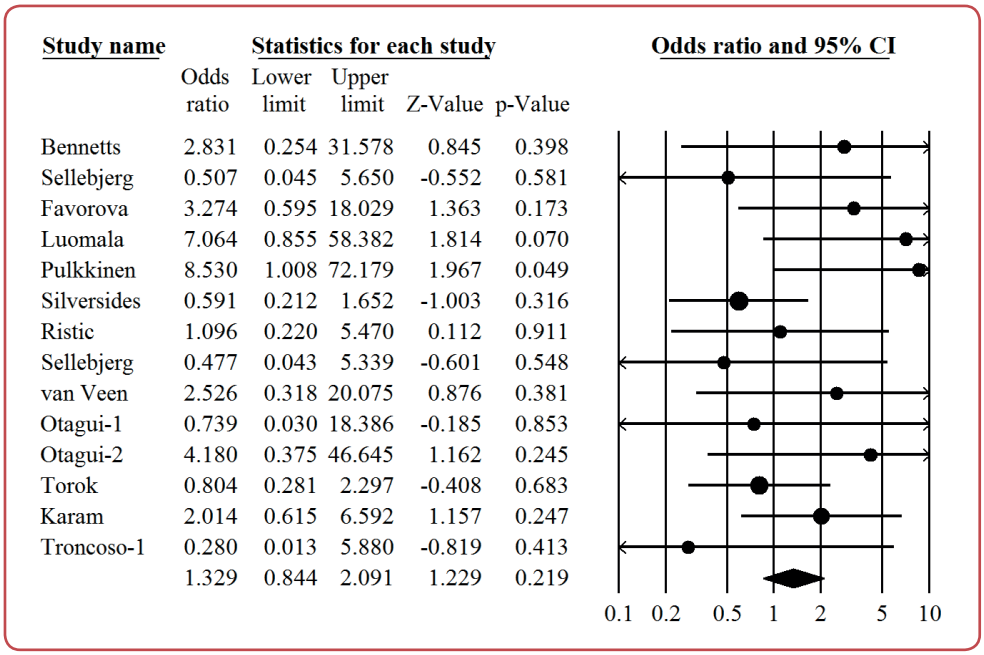

Figure 4: Forest plot of the association between chemokine receptor 5 delta32 (CCR5-Δ32) polymorphism and multiple sclerosis risk. Assuming the Δ32 allele act as a recessive allele

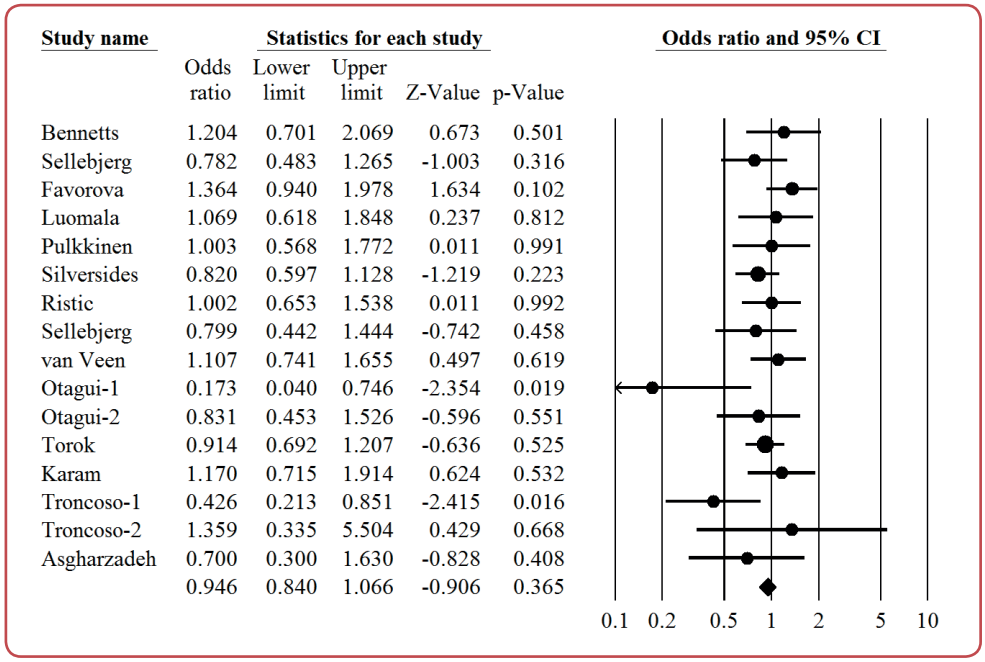

Figure 5: Forest plot of the association the Δ32 allele and multiple sclerosis risk

Discussion

Several original articles reporting the association between rs333 and susceptibility to MS,[18-21, 23-25, 27-29, 33-36] with controversial results. As mentioned in the Introduction, there is a meta-analysis on this topic,[37] but it did not include all eligible studies for analysis. Considering that the analysis suffers from the authors' inaccuracy, the current study was conducted. No association was found between rs333 and MS.

There have been several meta-analyses of the association between rs333 and the risk of various autoimmune diseases, with inconsistent results. Susceptibility to systemic lupus erythematosus[13, 14] and Sjögren's syndrome[14] is not associated with *CCR5*-Δ32, while the other diseases showed significant associations. However, in these cases, the nature of the associations is not similar, eg the variant allele has a protective effect on the risk of rheumatoid arthritis, juvenile idiopathic arthritis[47] and type 1 diabetes,[16] whereas the variant allele significantly increases the risk of Behçet's disease.[16, 48] It might be concluded that the *CCR5* gene and its Δ32 allele are not so important genetic variation for at least in susceptibility to autoimmune diseases.

Previously have been suggested that *CCR5* does not play an essential role in the context of immune cell migration into the central nervous system in MS.[33] The present finding supports this suggestion.

The fact that the rs333 polymorphism is not associated with the risk of MS does not mean that the *CCR5* gene and its polymorphism (rs333) have no effect on other aspects of the disease, such as age at onset and response to treatment. It should be noted that patients with MS who carry the Δ32 allele have an age at onset that is approximately 3 years later than patients who do not carry the Δ32 allele.[49] Other evidence suggests that the Δ32 allele may contribute to a slower rate of MS progression.[50] It has recently been reported that five autoimmune diseases, including multiple sclerosis, share a gene set of 12 polymorphic protein-coding genes, suggesting that they have a common genetic basis.[51] The *CCR5* gene is not only absent from the common gene set, but also from polymorphic genes associated with five autoimmune diseases. This indicates that polymorphisms of this gene were not associated with susceptibility to at least five autoimmune diseases studied using the GWAS database. The present finding that the rs333 was not associated with the risk of MS confirms the above study.

Current meta-analysis has a number of limitations. First, only English-language studies were included in the analysis, which means that related published reports in other languages were overlooked, which may lead to selection bias of eligible studies. Second, almost all studies reported genotypes in both sexes. Therefore, the interaction of sex and rs333 polymorphism on MS susceptibility cannot be determined. Third, genotyping data on subclasses of MS were not available. Finally, due to missing data reported in the original articles included in this meta-analysis, the influences of other relevant components, such as age and lifestyle and their interactions with the polymorphism on MS susceptibility were not analysed.

Conclusion

This study suggests that the association between rs333 and MS susceptibility is not significant. Further studies with more participants should be done to determine the true association between the polymorphism and susceptibility to MS.

Ethics

This study was a secondary analysis based on the currently existing dataset and did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

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None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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Methodology: MS
Formal analysis: NA, MS
Data curation: NA, MS
Writing - original draft: NA, MS
Writing - review and editing: NA, MS

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