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Research article

Causal relationship between the immune phenotype of monocytes and myasthenia gravis: A Mendelian randomization study

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ABSTRACT

Background: Monocytes play an essential role in developing autoimmune diseases; however, their association with myasthenia gravis (MG) development is unclear.
Methods: We performed a two-sample Mendelian randomization analysis to assess the causal relationship between monocyte-associated traits and MG, reviewing summary statistics of genome-wide association studies (GWAS).
Results: Using the inverse variance weighted method, the following were found to be causally associated with MG: HLA-DR on monocytes (OR, 1.363; 95% CI, 1.158–1.605; $P = 2E\text{-}04$), HLA-DR on $CD14^+$ monocytes (OR, 1.324; 95% CI, 1.183–1.482; $P = 1.08E\text{-}06$), HLA-DR on $CD14^+CD16^-$ monocytes (OR, 1.313; 95% CI, 1.177–1.465; $P = 1.07E\text{-}06$), CD40 on monocytes (OR, 1.135; 95% CI, 1.012–1.272; $P < 0.05$), CD40 on $CD14^+CD16^-$ monocytes (OR, 1.142; 95% CI, 1.015–1.285; $P < 0.05$), CD40 on $CD14^+CD16^+$ monocytes (OR, 1.142; 95% CI, 1.021–1.278; $P < 0.05$), CD64 on $CD14^+CD16^+$ monocytes (OR, 1.286; 95% CI, 1.019–1.623; $P < 0.05$).
Conclusions: The present study suggests a causal relationship between the upregulation of CD40, HLA-DR, and CD64 on monocytes and the development of MG. Altered monocyte function may potentially be a risk factor for MG and a therapeutic target.

1. Introduction

Myasthenia gravis (MG) is an autoimmune disease caused by autoantibodies affecting the postsynaptic membrane of the neuromuscular junction. It is clinically characterized by weakness and fatigue of the skeletal and extraocular muscles [1]. The primary pathogenic autoantibodies are those against the acetylcholine receptor (AChR), muscle-specific kinase (MUSK), and lipoprotein-related protein 4 (LRP4) [2]. A nationwide study in China showed that, after adjusting for age and sex, the incidence of MG was 0.68 per 100,000, with a slightly higher incidence rate in women [3]. The age of onset for AChR-related myasthenia gravis is bimodal, with peak onset occurring in young adults around the age of 30 years, and steadily increasing with age beyond 50. This also

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coincides with the peak onset in females, which is typical of many autoimmune diseases. However, the incidence of late-onset myasthenia gravis (LOMG) is slightly higher in males [4,5]. Based on clinical manifestations, antibody expression, and the presence or absence of a thymoma, MG can be classified into ocular myasthenia gravis, early-onset myasthenia gravis (EOMG, <50 years old), LOMG (≥50 years old), thymoma, and MUSK-associated types, LRP4-associated, and seronegative myasthenia gravis clinical subtypes [6]. Thymic follicular hyperplasia is frequent, though not a prerequisite in patients with EOMG, and often responds to thymectomy. Female cases outnumber male cases by a ratio of 3:1. EOMG is associated with HLA-DR3, HLA-B8, and other autoimmune risk genes. Moreover, all autoimmune diseases have been more widely reported in relatives of patients in the myasthenia gravis subgroup [7,8]. Thymic hyperplasia occurs rarely in patients with LOMG, and such patients most often do not respond to thymectomy. The disease is slightly more prevalent in men than women, and HLA correlates weakly with HLA- DR2, HLA-b7, and HLA-drb1 *15:01 [9]. Our previous study on MG suggests that there is a significant peak in the incidence of LOMG in women aged 60–70 years and men aged 70–80 years [10]. However, there is a distinct lack of research on this phenomenon, making it difficult to identify appropriate interventions. Therefore, the susceptibility factors of MG and the mechanism of its immune disorder have become the bottleneck in this field, and it is of great theoretical and clinical significance to study and solve this critical scientific problem.

Although MG is primarily defined as an antibody-mediated, T cell-dependent, complement-involved autoimmune disease, numerous other immune cells are essential in contributing to its pathogenesis. The presence of a host of autoantibodies and autoreactive B and T cells suggests that the adaptive immune system is vital for MG pathogenesis. However, this cannot fully explain the development of autoimmune diseases, as the innate immune response is undoubtedly involved [11,12]. Therefore, monocytes, fundamental to the intrinsic immune system (especially with their interaction with adaptive immunity), must be investigated to ascertain the inception of such autoimmune diseases, inflammatory cell infiltration in target organs, and release of cytokines and chemokines [13]. As they develop in the bone marrow and enter the bloodstream, monocytes enter tissues for further migration and differentiation to promote inflammatory responses or subsidence [14]. Several studies have shown that monocytes can take up, process, and present antigens *in vivo*. However, it is still debated whether monocytes play a significant role in T-cell initiation when compared to cDC presentation of antigens, which seems to depend on the inflammatory environment [15]. More than 100 differential gene expressions have been detected in peripheral monocytes of MG patients. These gene expressions can impaired monocyte function

Table 1
Details of data sources included in the study.Abbreviations: SNPs, single nucleotide polymorphism.

Phenotypes	GWAS ID	Year	Sample size	Ancestor	Number of SNPs	Pubmed ID
Monocyte count	ebi-a-GCST004625	2016	170,721	European	29,166,012	27863252
Monocyte percentage of white cells	ebi-a-GCST004609	2016	170,494	European	29,165,229	27863252
Monocyte Absolute Count	ebi-a-GCST90001583	2020	3,629	European	15,038,157	32929287
CD14 + CD16$^-$ monocyte Absolute Count	ebi-a-GCST90001582	2020	3,629	European	15,038,157	32929287
CD14$^+$ CD16$^-$ monocyte %monocyte	ebi-a-GCST90001586	2020	3,629	European	15,038,157	32929287
CD14$^+$ CD16$^+$ monocyte Absolute Count	ebi-a-GCST90001580	2020	3,629	European	15,038,157	32929287
CD14$^+$ CD16$^+$ monocyte %monocyte	ebi-a-GCST90001585	2020	3,629	European	15,038,157	32929287
CD14$^-$ CD16$^+$ monocyte Absolute Count	ebi-a-GCST90001579	2020	3,629	European	15,038,157	32929287
CD14$^-$ CD16$^+$ monocyte %monocyte	ebi-a-GCST90001584	2020	3,629	European	15,038,157	32929287
HLA DR on monocyte	ebi-a-GCST90002010	2020	3,629	European	15,034,296	32929287
HLA DR on CD14$^+$ monocyte	ebi-a-GCST90001991	2020	3,629	European	15,034,296	32929287
HLA DR on CD14$^+$ CD16$^-$ monocyte	ebi-a-GCST90001988	2020	3,629	European	15,034,296	32929287
HLA DR on CD14$^+$ CD16$^+$ monocyte	ebi-a-GCST90002007	2020	3,618	European	15,030,660	32929287
HLA DR on CD14$^-$ CD16$^+$ monocyte	ebi-a-GCST90001984	2020	3,621	European	15,029,878	32929287
PDL-1 on monocyte	ebi-a-GCST90002002	2020	3,629	European	15,034,296	32929287
PDL-1 on CD14$^+$ CD16$^-$ monocyte	ebi-a-GCST90001993	2020	3,629	European	15,034,296	32929287
PDL-1 on CD14$^+$ CD16$^+$ monocyte	ebi-a-GCST90001998	2020	3,618	European	15,030,660	32929287
PDL-1 on CD14$^-$ CD16$^+$ monocyte	ebi-a-GCST90001999	2020	3,621	European	15,029,878	32929287
CD40 on monocytes	ebi-a-GCST90001985	2020	3,629	European	15,034,296	32929287
CD40 on CD14$^+$ CD16$^-$ monocyte	ebi-a-GCST90001980	2020	3,629	European	15,034,296	32929287
CD40 on CD14$^+$ CD16$^+$ monocyte	ebi-a-GCST90001981	2020	3,618	European	15,030,660	32929287
CD40 on CD14$^-$ CD16$^+$ monocyte	ebi-a-GCST90001989	2020	3,621	European	15,029,878	32929287
CD80 on monocyte	ebi-a-GCST90002039	2020	2,850	European	14,821,110	32929287
CD86 on monocyte	ebi-a-GCST90001905	2020	2,850	European	14,821,110	32929287
CD62L on monocyte	ebi-a-GCST90001834	2020	2,848	European	13,916,277	32929287
CD64 on monocyte	ebi-a-GCST90002006	2020	3,622	European	15,031,257	32929287
CD64 on CD14$^+$ CD16$^-$ monocyte	ebi-a-GCST90001987	2020	3,622	European	15,031,257	32929287
CD64 on CD14$^+$ CD16$^+$ monocyte	ebi-a-GCST90002011	2020	3,611	European	14,109,235	32929287
CD64 on CD14$^-$ CD16$^+$ monocyte	ebi-a-GCST90001990	2020	3,614	European	15,026,836	32929287
CX3CR1 on monocyte	ebi-a-GCST90001995	2020	3,590	European	15,023,496	32929287
CX3CR1 on CD14$^+$ CD16$^-$ monocyte	ebi-a-GCST90001997	2020	3,590	European	15,023,496	32929287
CX3CR1 on CD14$^+$ CD16$^+$ monocyte	ebi-a-GCST90001996	2020	3,579	European	15,019,836	32929287
CX3CR1 on CD14$^-$ CD16$^+$ monocyte	ebi-a-GCST90002012	2020	3,582	European	15,019,052	32929287
CCR2 on monocyte	ebi-a-GCST90002017	2020	2,850	European	14,821,110	32929287
CCR2 on CD14$^+$ CD16$^-$ monocyte	ebi-a-GCST90002004	2020	3,629	European	15,034,296	32929287
CCR2 on CD14$^+$ CD16$^+$ monocyte	ebi-a-GCST90001992	2020	3,618	European	15,030,660	32929287
CCR2 on CD14$^-$ CD16$^+$ monocyte	ebi-a-GCST90001982	2020	3,621	European	15,029,878	32929287
Myasthenia gravis	GCST90093061	2022	38,243	US and Italian	24,006,245	35074870

and further reduced expression of genes associated with inflammatory regression, which may contribute to the chronicity of the disease [16]. A recent study showed a significant increase in the frequency of VISTA^{+}CD14^{+} monocytes in patients with MG [17]. Therefore, the observed alterations in the number and function of monocytes in patients with MG are likely to be closely associated with the disease's development.

Mendelian randomization (MR) is an advancing epidemiological approach that uses genetic variations as instrumental variables to infer whether exposure (or risk) factors have causal effects on health outcomes [18]. It relies on the natural random classification of genetic variation during meiosis to produce a random distribution of genetic variation in a population. Compared with randomized trials, MR avoids using interventions to test a hypothesis to reduce the effects of confounding factors and selection bias [19]. It provides more reliable results by genetically inferring the correlation between the exposure and outcome.

Therefore, the improved methodology of MR was implemented to examine the causal relationship between monocytes and MG and to investigate the pathogenesis of MG.

2. Materials and methods

2.1. Data sources

As the data source, publicly available genome-wide association study (GWAS) data abstracts were gathered. The details of the datasets relevant to the GWAS are shown in Table 1.

Monocyte counts and monocyte percentages of white blood cells were obtained from a large GWAS of patients of European ancestry. This GWAS includes associations of 29.5 million genetic variants with 36 characteristics of erythrocytes, leukocytes, and platelets in 173,480 participants of European origin [20]. The remaining monocyte phenotypes were derived from another GWAS, the largest published study on peripheral blood immune phenotypes, which analysed the association of 757 immune cell traits with natural genetic variation in a population of 7,313 Sardinian descent [21].

Furthermore, the MG data were derived from the most extensive GWAS published to date, which included US and Italian patients with *anti*-AChR^{+} MG. 1,873 patients with 36,370 age- and sex-matched controls were included, while patients with positive MuSK test results were excluded [22]. Ethical approval was obtained for all original studies, and informed consent was obtained from all subjects. Therefore, all the anonymized patient data used for this GWAS study comply with the standard ethical guidelines.

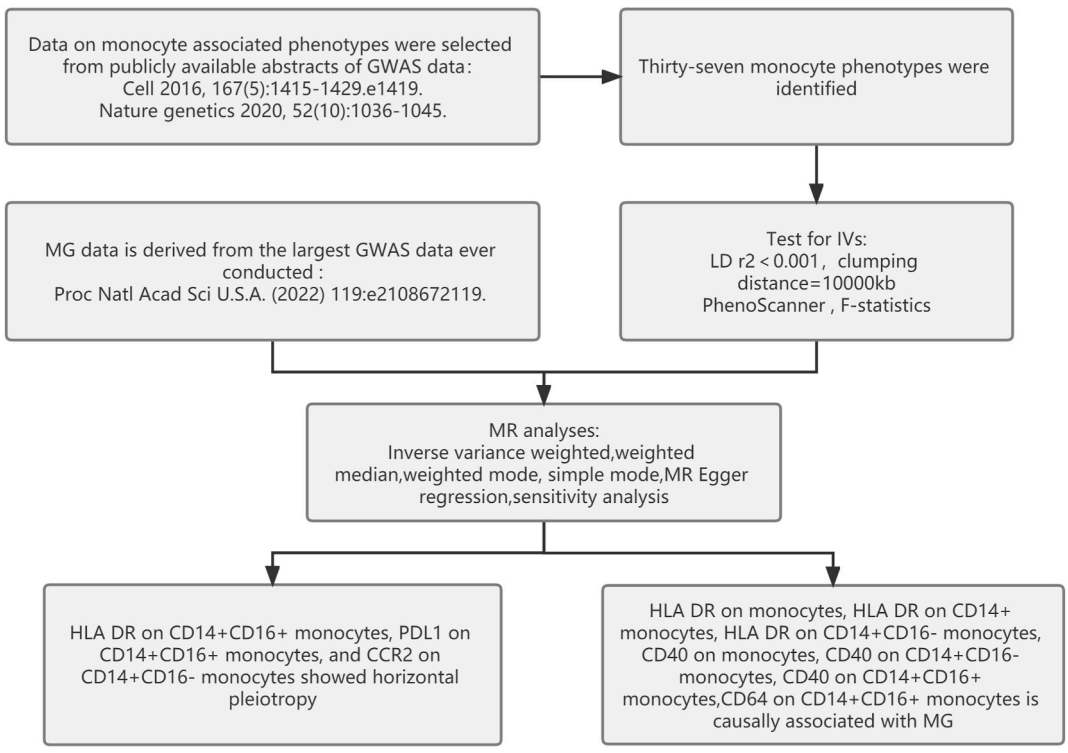

Fig. 1. Study flow diagram. Two-sample Mendelian randomization analyses were performed using GWAS data from publicly available databases to assess the causal relationship between MG and monocytes, and sensitivity analyses were performed.

2.2. Selection of instrumental variables

First, single nucleotide polymorphisms (SNPs) associated with monocyte immune traits were extracted at the genome-wide significance level. Single nucleotide polymorphisms (SNPs) with significance levels of $P < 5.00E-8$ were selected for assessing the monocyte count, monocyte percentage of white cells, HLA-DR on $CD14^+$ monocytes, HLA-DR on $CD14^+$ $CD16^-$ monocytes, HLA-DR on $CD14^-CD16^+$ monocytes, CD40 on monocytes, CD40 on $CD14^+$ $CD16^-$ monocytes, CD40 on $CD14^+$ $CD16^+$ monocytes, CD80 on monocytes, CD64 on monocytes, and CD64 on $CD14^+$ $CD16^-$ monocytes. To avoid inaccurate results due to a few SNPs, the significance threshold of the SNPs for the remaining monocyte traits was relaxed to 5.00E-6. We set the linkage disequilibrium coefficient (r^2) to 0.001 and the width of the linkage disequilibrium region to 10,000 kb to ensure each SNP's independence and exclude the effect of gene pleiotropy on the results [23,24]. Palindromic SNPs with intermediate allele frequencies from instrumental variables (IVs) were excluded. The IVs were then coordinated to ensure that their association effects were associated with the identical alleles in terms of exposure and outcomes. Finally, according to MR's third hypothesis, genetic variation cannot be related to possible confounders; therefore, we used PhenoScanner to verify whether the said incorporated SNPs were associated with other confounders [25]. We also calculated the F-statistic for each IV to assess the strength of the association with exposure and included IVs with an F-statistic >10 [26,27]. The F statistic is calculated as $F = \beta^2/SE^2$, where β represents the effect on exposure risk, and SE is the standard error [28,29].

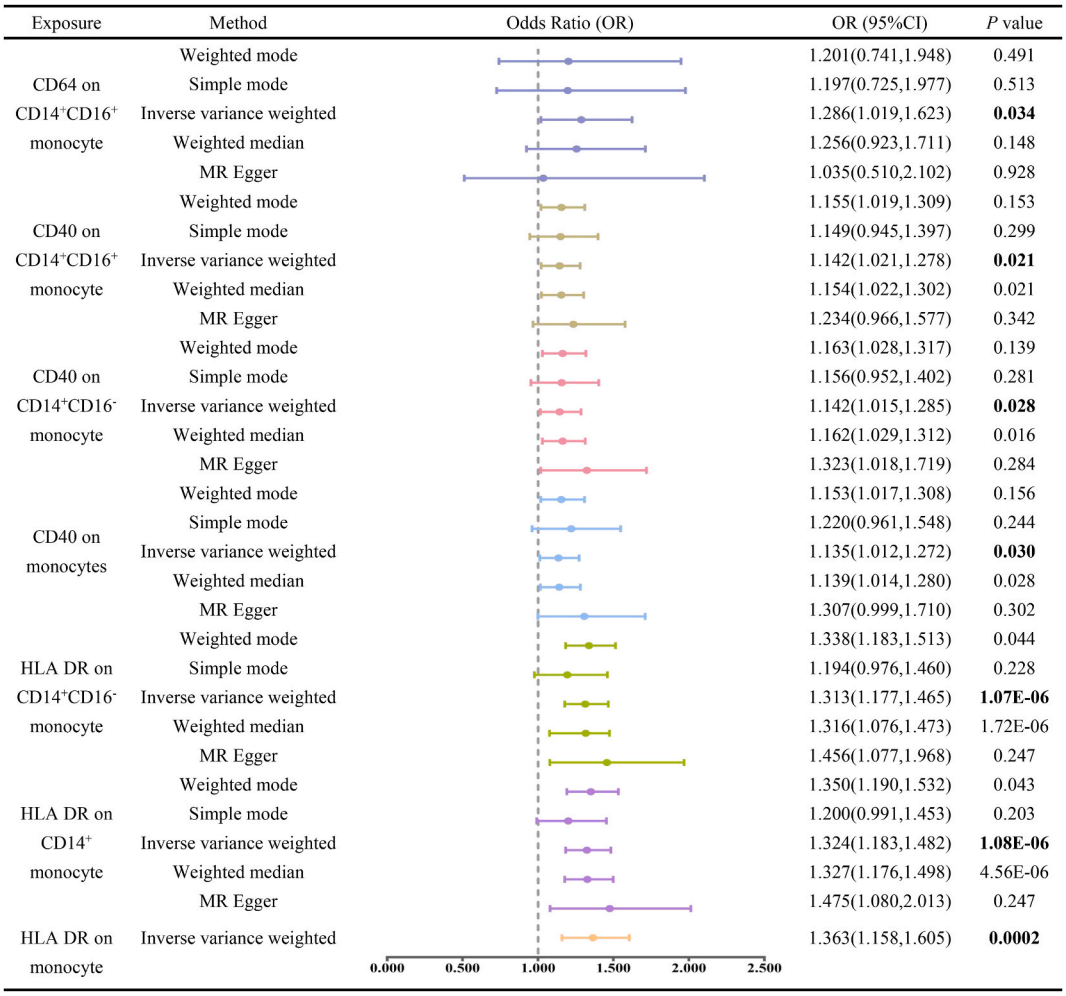

Fig. 2. Certain traits of monocytes are risk factors for myasthenia gravis. Using the inverse variance weighted method, the following were found to be causally associated with MG: HLA-DR on monocytes (OR, 1.363; 95% CI, 1.158–1.605; $P = 2E-04$), HLA-DR on $CD14^+$ monocytes (OR, 1.324; 95% CI, 1.183–1.482; $P = 1.08E-06$), HLA-DR on $CD14^+CD16^-$ monocytes (OR, 1.313; 95% CI, 1.177–1.465; $P = 1.07E-06$), CD40 on monocytes (OR, 1.135; 95% CI, 1.012–1.272; $P < 0.05$), CD40 on $CD14^+CD16^-$ monocytes (OR, 1.142; 95% CI, 1.015–1.285; $P < 0.05$), CD40 on $CD14^+CD16^+$ monocytes (OR, 1.142; 95% CI, 1.021–1.278; $P < 0.05$), CD64 on $CD14^+CD16^+$ monocytes (OR, 1.286; 95% CI, 1.019–1.623; $P < 0.05$).

2.3. Two-sample MR analysis

To investigate the causal effect of exposure on the outcome, we conducted MR analysis using five methods, inverse variance weighting (IVW), weighted median, weighted mode, simple mode, and MR-Egger regression) to verify the causal relationship between exposure (monocyte-related phenotype) and outcome (MG), using SNPs as the instrumental variable. However, these methods make different assumptions at the expense of reduced statistical power. Several comprehensive sensitivity analyses were conducted to exclude possible violations of the MR hypothesis (i.e., heterogeneity and pleiotropy). Cochran's Q statistic tested heterogeneity; a p-value <0.05. Was considered significant heterogeneity [30]. If there was substantial heterogeneity, we used the MR-PRESSO method to detect outliers (Nb Distribution = 10,000), remove them, and reanalyze them [31]. We also performed the MR-Egger intercept test to clarify whether there were horizontal pleiotropic effects in this MR analysis. If the intercept term in the MR-Egger intercept analysis was statistically significant, it indicates that the study has potential horizontal pleiotropic effects [32]. We also performed leave-one-out analyses to assess whether the MR analysis results were driven by a single SNP [21] (Fig. 1). The study was conducted using *R* software (version 4.2.2), and MR analysis was performed using the TwoSampleMR package (version 0.5.6).

3. Results

3.1. Instrumental variables

The analysis identified 37 monocytic traits to have a causal relationship between monocytes and MG. The details of the SNPs associated with each trait are shown in Supplementary Table S1. The F-statistics for all instrumental variables were above ten, indicating the absence of a weak instrumental bias (Supplementary Table S1).

Table 2
The result of sensitivity analyses of MR. Cochran's Q statistic showed heterogeneity in SNPs for monocyte count and monocyte percentage of white blood cells ($P < 0.05$). MR Egger intercept test showed horizontal pleiotropy in the study of HLA-DR on CD14 + $CD16^+$ monocyte, PD-L1 on CD14 + $CD16^+$ monocyte, and CCR2 on CD14 + $CD16^-$monocyte.

Exposure	IVW Estimates		MR-Egger Pleiotropy Test	
	Cochran's Q	p-value	MR-Egger Intercept	p-value
Monocyte count	200.798	**8.47E-05**	0.009	0.314
Monocyte percentage of white cells	199.361	**0.0009**	−0.003	0.686
Monocyte Absolute Count	6.797	0.815	0.013	0.538
CD14 + $CD16^-$ monocyte Absolute Count	6.702	0.753	0.031	0.37
$CD14^+$ $CD16^-$ monocyte %monocyte	16.980	0.15	0.024	0.3
$CD14^+$ $CD16^+$ monocyte Absolute Count	12.276	0.056	0.023	0.635
$CD14^+$ $CD16^+$ monocyte %monocyte	20.959	0.103	0.065	0.104
$CD14^-$ $CD16^+$ monocyte Absolute Count	8.390	0.678	−0.0003	0.994
$CD14^-$ $CD16^+$ monocyte %monocyte	5.188	0.737	−0.004	0.909
HLA DR on monocyte	1.540	0.215	NA	NA
HLA DR on $CD14^+$ monocyte	0.912	0.634	−0.052	0.599
HLA DR on $CD14^+$ $CD16^-$ monocyte	0.899	0.638	−0.051	0.601
HLA DR on $CD14^+$ $CD16^+$ monocyte	12.813	0.171	−0.067	**0.037**
HLA DR on $CD14^-$ $CD16^+$ monocyte	1.930	0.381	0.759	0.436
PDL-1 on monocyte	7.887	0.343	0.018	0.643
PDL-1 on $CD14^+$ $CD16^-$ monocyte	8.943	0.177	0.027	0.615
PDL-1 on $CD14^+$ $CD16^+$ monocyte	15.194	0.295	−0.048	**0.041**
PDL-1 on $CD14^-$ $CD16^+$ monocyte	18.699	0.067	0.070	0.179
CD40 on monocytes	1.705	0.426	−0.088	0.460
CD40 on $CD14^+$ $CD16^-$ monocyte	1.523	0.467	−0.084	0.434
CD40 on $CD14^+$ $CD16^+$ monocyte	0.544	0.762	−0.044	0.613
CD40 on $CD14^-$ $CD16^+$ monocyte	11.774	0.464	−0.003	0.942
CD80 on monocyte	3.349	0.501	−0.055	0.371
CD86 on monocyte	4.433	0.489	0.016	0.673
CD62L on monocyte	10.136	0.256	0.060	0.098
CD64 on monocyte	1.058	0.958	−0.076	0.552
CD64 on $CD14^+$ $CD16^-$ monocyte	1.058	0.958	−0.081	0.541
CD64 on $CD14^+$ $CD16^+$ monocyte	5.183	0.394	0.037	0.557
CD64 on $CD14^-$ $CD16^+$ monocyte	15.938	0.253	0.010	0.743
CX3CR1 on monocyte	20.663	0.080	−0.020	0.667
CX3CR1 on $CD14^+$ $CD16^-$ monocyte	20.131	0.092	−0.016	0.699
CX3CR1 on $CD14^+$ $CD16^+$ monocyte	13.736	0.470	0.008	0.770
CX3CR1 on $CD14^-$ $CD16^+$ monocyte	7.297	0.505	0.096	0.237
CCR2 on monocyte	10.430	0.404	−0.039	0.443
CCR2 on $CD14^+$ $CD16^-$ monocyte	16.023	0.099	0.060	**0.020**
CCR2 on $CD14^+$ $CD16^+$ monocyte	12.953	0.794	0.006	0.780
CCR2 on $CD14^-$ $CD16^+$ monocyte	11.315	0.417	−0.001	0.962

3.2. MR analysis of the causal relationships between monocytes and MG

Analysis of the selected instrumental variables revealed certain monocyte traits positively correlated with MG. Using a random-effects IVW method, we found a causal association of MG with: HLA-DR on monocytes (OR, 1.363; 95% CI, 1.158–1.605; $P = 2E\text{-}04$), HLA-DR on $CD14^+$ monocytes (OR, 1.324; 95% CI, 1.183–1.482; $P = 1.08E\text{-}06$), HLA-DR on $CD14^+CD16^-$ monocytes (OR, 1.313; 95% CI, 1.177–1.465; $P = 1.07E\text{-}06$), CD40 on monocytes (OR, 1.135; 95% CI, 1.012–1.272; $P < 0.05$), CD40 on $CD14^+CD16^-$ monocytes (OR, 1.142; 95% CI, 1.015–1.285; $P < 0.05$), CD40 on $CD14^+CD16^+$ monocytes (OR, 1.142; 95% CI, 1.021–1.278; $P < 0.05$), CD64 on $CD14^+CD16^+$ monocytes (OR, 1.286; 95% CI, 1.019–1.623; $P < 0.05$). This suggested that monocytes may play a dangerous role in the development of MG (Fig. 2, Supplementary Table S2). Several other MR methods also showed that the estimates of the causal effect of monocytes on MG were almost identical to those obtained using IVW (Fig. 2, Supplementary Table S2). The remaining monocyte immunophenotypes were unrelated to MG ($P > 0.05$). Single-cell sequencing of MG patients revealed that monocytes exhibited more heterogeneity. Differential gene analysis of $CD14^+$ monocytes showed that MG patients expressed high levels of inflammatory markers S100A4, S100A8, S100A9, S100A10, and S100A12 and that the most pronounced pathway changes in MG patients were in inflammation-related pathways, including MAPK family signalling, TNF signalling, TLR4, interferon, and interleukin signalling, suggesting that inflammatory pathways are highly activated in monocytes [33]. Therefore, monocytes also play a crucial role in the pathological process of MG.

3.3. Sensitivity analysis

Cochran's Q statistic showed heterogeneity in SNPs for monocyte count and monocyte percentage of white blood cells ($P < 0.05$) but no heterogeneity in the remaining monocyte phenotypes ($P > 0.05$). For monocyte phenotypes with heterogeneity, the outliers were examined using the MR-PRESSO method (Nb Distribution = 10,000). The analysis was repeated after excluding the outliers, which still suggested heterogeneity. MR Egger intercept test showed horizontal pleiotropy in the study of HLA-DR on $CD14^+CD16^+$ monocyte, PD-L1 on $CD14^+CD16^+$ monocyte, and CCR2 on $CD14^+CD16^-$ monocyte. The results of the IVW method could not be explained due to horizontal pleiotropy, which violates the second MR hypothesis. The MR-Egger intercept test for the remaining monocyte phenotypes did not suggest any evidence of horizontal pleiotropy ($P > 0.05$). The leave-one-out analysis showed that the removal of SNPs did not fundamentally affect the results, indicating that the results were stable and reliable (Table 2 and Fig. 3).

4. Discussion

Monocytes are cells in the circulating blood, accounting for approximately 10% of peripheral blood leukocytes in humans and 4% in mice. Circulating mononuclear cells consist of distinct subpopulations with functional properties before reaching the inflamed tissue. Monocytes play a crucial role in innate immunity by promoting immunomodulation, inflammation, and tissue repair through

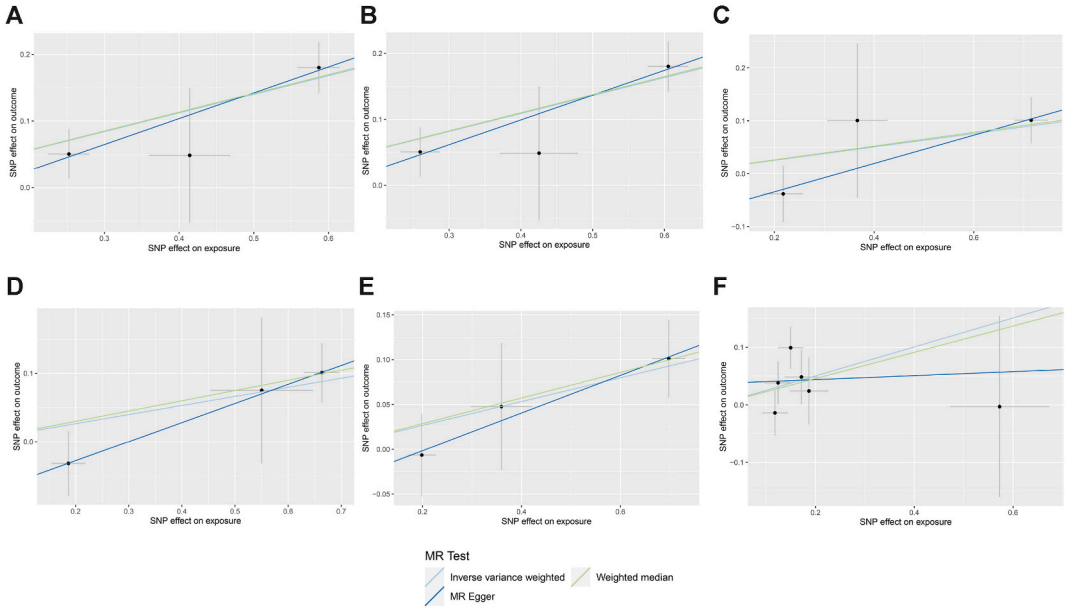

Fig. 3. Scatter plot of MR Analysis. (A) Scatter plots of relationship between MG and HLA DR on $CD14^+$ monocyte. (B) Scatter plots of relationship between MG and HLA DR on $CD14^+CD16^-$ monocyte. (C) A. Scatter plots of relationship between MG and CD40 on monocytes. (D) Scatter plots of relationship between MG and CD40 on $CD14^+CD16^-$ monocyte. (E) Scatter plots of relationship between MG and CD40 on $CD14^+CD16^+$ monocyte. (F) Scatter plots of relationship between MG and CD64 on $CD14^+CD16^+$ monocyte.

phagocytosis. They also participate in antigen presentation and the production of cytokines and chemokines, which are involved in developing many autoimmune diseases [11,13]. Monocytes can be divided into three subpopulations based on CD14 and CD16 expression: classical $CD14^+CD16^-$ monocytes (≥90%), intermediate $CD14^+CD16^+$ monocytes, and non-classical $CD14^-CD16^+$ monocytes. There appears to be a developmental relationship between these three subpopulations [34]. Different subpopulations have different functions. Classic $CD14^+CD16^-$ monocytes are involved in various immune responses, such as inflammation and tissue repair. Intermediate monocytes with a $CD14^+CD16^+$ phenotype highly express TLR2, TLR4, and HLA-DR and have the highest antigen-presenting capacity. $CD14^-CD16^+$ non-classical monocytes are called "patrol" monocytes and can stimulate the proliferation of $CD4^+$ T cells [35].

Previous studies have shown that monocytes are associated with various autoimmune diseases. For instance, Sümegi et al. (2005) showed that absolute monocyte counts were similar in patients with systemic lupus erythematosus (SLE) and healthy controls. In contrast, the ratio and total number of $CD14^-CD16^+$ monocytes were significantly higher in patients with SLE. Furthermore, hormone therapy dose-dependently downregulated the percentage and number of $CD14^-CD16^+$ monocytes [36]. There was a significant correlation between the ratio of $CD14^+CD16^+$ monocytes and the clinical activity index in patients with inflammatory bowel disease (IBD) [37], and a substantial increase in peripheral $CD14^+CD16^+$ monocytes was observed in patients with active Crohn's disease, especially in patients with colonic involvement and a high disease activity index [38]. In the context of MG, the immune system erroneously targets elements of the neuromuscular junction, primarily the acetylcholine receptors, resulting in muscle weakness. While a significant portion of research has concentrated on the involvement of B cells and autoantibodies in MG, the role of monocytes and their different subsets is becoming an increasingly intriguing study area. Recent investigations have indicated that the particular monocyte subset may participate in the development of MG, potentially influencing the autoimmune response and inflammation in affected individuals.

Nevertheless, the precise role of these cells and their subsets, including the more recently defined $Slan^+$ and Tie2, in the immunopathogenesis of MG remains unclear. Recent investigations have indicated those particular monocyte subsets may participate in the development of MG, potentially influencing the autoimmune response and inflammation in affected individuals. It has been observed that all subpopulations of monocytes are reduced in MG patients, including classic, intermediate, and atypical monocytes, and there is an overall reduction in monocyte activity in MG patients [39,40]. Researchers have founds that monocytes 3 (FCGR3B monocytes) may be associated with hypercellular kinasemia in muscle weakness diseases [41]. New monocyte subpopulations such as neutrophil-like Ly6CHi monocytes, SatM, and $CD209^+$ monocytes emerge under both inflammatory and healthy conditions. These cells have enhanced pro-inflammatory, pro-necrotic, and antigen-presenting capabilities compared to steady-state Ly6CHi monocytes [42–44]. A recent study reported an increase in the frequency of $VISTA^+CD14^+$ monocytes and HLA-DR expression in $VISTA^+CD14^+$ monocytes in patients with MG [17]. Additional studies have shown that VISTA expression can activate $CD14^+$ monocytes and promote inflammatory responses [45]. This suggests that $CD14^+$ monocytes are involved in the development of MG. Nevertheless, the precise role of these cells and their subsets, including the more recently defined Slan+ and Tie2, in the immunopathogenesis of MG remains unclear. This study found a positive correlation between MG and HLA-DR on monocytes, HLA-DR on $CD14^+$ monocytes, HLA-DR on $CD14^+CD16^-$ monocytes, CD40 on monocytes, CD40 on $CD14^+CD16^-$ monocytes, CD40 on $CD14^+CD16^+$ monocytes, and CD64 on $CD14^+CD16^+$ monocytes, using two-sample MR analysis. These results suggest that activation of monocyte function plays a vital role in the development of MG. In contrast, changes in the number of monocytes may not be involved in MG development. So far, it is the first study to elucidate the causal relationship between monocytes and MG from the perspective of genetic variation using a two-sample MR analysis.

There are several physiological differences between males and females, most notably their role in reproduction, and the concentration of hormones [46]. More than three-quarters of people with autoimmune diseases are women [47], though ankylosing spondylitis, vasculitis, and Goodpasture's syndrome do occur predominantly in men [48]. Changes in monocytes are also associated with sex. Under physiological conditions, monocyte counts have been reported to be consistently elevated in males at all stages of life [49–51], and the proportion of nonclassical monocytes differs between sexes [52]. These differences in monocyte subpopulations can be attributed to the effects of estrogen and other sex hormones, with increases in estrogen decreasing the number of monocytes, thus supporting the observation that monocyte counts tend to be higher in men [53,54]. In addition, there are sex differences in monocyte cytotoxic activity and cytokine production [50,55]. However, female hormones are not responsible for this effect [56]. Thus, the effect of estrogen on monocyte function may only become apparent in response to specific stimuli.

In previous studies, monocyte HLA-DR (mHLA-DR) expression levels reflected the monocytes' pro- and anti-inflammatory functional status [57]. Low mHLA-DR expression can be used as a marker of sepsis-induced immunosuppression [58,59]. The reduced expression of mHLA-DR represents a decrease in the monocyte antigen presentation capacity, further explaining the phenomenon of endotoxin tolerance characterized by altered function in sepsis [60,61]. In addition to sepsis, mHLA-DR has become a popular immunosurveillance tool in other clinical areas for monitoring mortality, secondary infections, and worsening events of cancer recurrence [57,62]. Thus, mHLA-DR appears to be a valid indicator of persistent immune activation and autoimmunity [61]. Significantly increased HLA-DR levels in $CD14^+$ monocytes have been reported in multiple sclerosis (MS) patients, and this increase is most pronounced in $CD16^+$ monocytes [63]. CD14 and HLA-DR expression increase with disease duration and severity in amyotrophic lateral sclerosis (ALS) [64]. This is consistent with our findings in MG. We demonstrated that the increased levels of HLA-DR on monocytes and $CD14^+$ monocytes are positively associated with the development of MG, especially with HLA-DR on $CD14^+CD16^-$ monocytes being more representative. In the present study, we considered that the elevated increased levels of monocyte HLA-DR may represent overactivation of the immune system, thereby increasing the risk of developing MG.

CD40 is a transmembrane cell surface receptor, a co-stimulatory molecule of the tumor necrosis factor family expressed on various immune and non-immune cells. CD40 and its ligands regulate humoral and cellular immunity [65–67]. Studies have shown that CD40

expression on the surface of monocytes is closely associated with autoimmune diseases. For example, CD40 expression on $CD14^+$ monocytes significantly increased in patients with MS [63]. In renal biopsies from patients with lupus nephritis, the expression level of monocyte CD40 was significantly upregulated [68]. Treatment of human primary monocytes with granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, or IFN-γ induces the expression of CD40. CD40, in turn, induces effector functions in monocytes [69]. However, the relationship between CD40 expression in monocytes and MG has not yet been confirmed. The MR method of this study allowed us to identify a causal relationship between CD40 expression on monocytes and MG. We further investigated CD40 expression in different monocyte phenotypes. We found a causal relationship between MG and CD40 on $CD14^+CD16^-$ monocytes and CD40 on $CD14^+CD16^+$ monocytes, indicating that the CD40 increased expression levels on the surface of classical monocytes and intermediate monocytes were positively correlated with MG.

CD64 bridges humoral and cellular immunity, mainly expressed in innate immune cells. It is critical in cell phagocytosis, clearance of immune complexes, antigen presentation, and promoting inflammatory factor release [70]. Related studies have shown that CD64 on monocytes induces phagocytosis and promotes the secretion of proinflammatory cytokines by binding to the Fc portion of IgG antibodies [71]. CD64 acts as a high-affinity IgG Fc segment receptor that promotes the monocyte binding of antibodies and antigen-antibody immune complexes, thereby releasing inflammatory factors and promoting the development of autoimmune diseases [72]. Although the relationship between the expression levels and function of CD64 in monocytes and MG is currently unclear, our analysis showed that increased levels of CD64 on $CD14^+CD16^+$ monocytes was positively correlated with MG. This finding is consistent with those of the previous studies.

Viral infections trigger multiple immune response reactions in the body, which can lead to autoimmune diseases. MG has been found to be associated with a number of viral infections, such as Epstein-Barr virus (EBV), hepatitis E virus (HEV), West Nile virus (WNV) a human parvovirus B19 (HPVB19) [73]. On the one hand, this may be due to the fact that viral infections are associated with pathogenesis within the MG thymus [73]. Conversely, viral infections can also lead to elevated monocyte counts, in the course of which alterations in monocyte counts and function may be related to the pathogenesis of MG, which requires confirmation by future studies. Additionally, certain clinical agents for the treatment of MG also inhibit monocytes, which provides ideas for targeting monocytes for the treatment of myasthenia gravis [74].

The present study has limitations. First, this study mainly analysed the GWAS biobank of the European population and would not represent diverse and globally inclusive populations to represent the actual situation fully. Second, we should have specifically analysed the causal relationship between EOMG and LOMG and monocyte-related phenotypes according to age subgroups due to the need for more sufficient data. In addition, all patients with MG involved in this study were $AChR^+$. Patients with other antibody-positive or antibody-negative MG were excluded. Third, monocyte counts and percentages were heterogeneous when MR analysis was performed. Although we detected outliers using the MR-PRESSO method and reanalysed them after excluding them, heterogeneity was still suggested; therefore, the results must be interpreted cautiously. Similarly, there was horizontal pleiotropy in the analysis of HLA-DR on $CD14^+CD16^+$ monocytes, PD-L1 on $CD14^+CD16^+$ monocytes, and CCR2 on $CD14^+CD16^-$ monocytes in MG; we were therefore unable to interpret the results of the IVW approach. Additionally, the magnitude of the OR is not very high, and quite close to 1 for most markers. Fourth, it is well known that MG occurs more often in women. This factor may have influenced our results; however, we could not stratify the database by sex, and is one of the limitations of our study. Finally, MR analysis also has some inherent drawbacks, such as the inability to eliminate the influence of confounders. The accuracy of genetic instruments is essential for the validity of the MR approach, however, there remains the possibility of a weak instrument bias.

In conclusion, we found that the increased levels of CCR2, CX3CR1, and PD-L1 on monocytes and their subtypes may not be involved in MG development. Our results suggest that the increased levels of CD40, HLA-DR, and CD64 on monocytes contributes to the development of MG, presumably through their enhanced antigen-presenting ability and further interaction with autoreactive T cells, which in turn causes excessive activation of the autoimmune system and exacerbates MG progression. Further, this suggests that downregulating the antigen-presenting ability of monocytes to reduce the facilitation of autoimmune diseases may serve as a potential therapeutic target. It is important that these findings be confirmed by testing clinical specimens in future studies.

Data available statement

The authors confirm that the data supporting the findings of this study are available within the article.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The datasets generated during and analysed during the current study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Jing Dong: Writing – original draft, Software, Formal analysis, Data curation. **Rui-sheng Duan:** Writing – review & editing, Project administration, Funding acquisition. **Peng Zhang:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26741.

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