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Original Article

Mendelian Randomization Analysis Reveals the Causal Relationships Between Autoimmune Diseases and Idiopathic Thrombocytopenic Purpura and the Mediating Role of Immune Cells

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Abstract

Background: Idiopathic thrombocytopenic purpura (ITP) is related to multiple autoimmune diseases clinically, yet the causal relationship remains unclear. This study employed Mendelian randomization (MR) to explore the genetic causal relationship between autoimmune diseases and ITP and potential mediators in the European population.

Methods: Summary statistics of 10 common autoimmune diseases and ITP were extracted for analysis. Bidirectional two-sample MR and two-step MR were conducted.

Results: Multiple sclerosis (MS, inverse variance weighted [IVW]: odds ratio (OR) = 5.840E+16, false discovery rate (FDR) = 0.049), celiac disease (CeD, IVW: OR = 1.173, FDR = 0.023), systemic lupus erythematosus (SLE, IVW: OR = 1.068, FDR = 0.049), and autoimmune hyperthyroidism (AIH, IVW: OR = 1.265, FDR = 0.037) are risk factors for ITP. Rheumatoid arthritis (RA, IVW: OR = 1.112, FDR = 0.055) may be a potential risk factor. Crohn's disease (CD, IVW: OR = 0.816, FDR = 0.049), ulcerative colitis (UC, IVW: OR = 0.709, FDR = 0.042), and psoriasis (PsO, IVW: OR = 1.690E − 04, FDR = 0.042) are protective factors. No clear causal relationship between ankylosing spondylitis (AS, IVW: OR = 1.016, FDR = 0.553) and type 1 diabetes mellitus (T1DM, IVW: OR = 1.035, FDR = 0.577) and ITP. Immune cells act as mediators between CeD and ITP and CD and ITP.

Conclusion: This study clarified the relationship between some autoimmune diseases and ITP and the mediating role of immune cells.

Keywords: Celiac disease, Crohn's disease, idiopathic thrombocytopenic purpura, immune cell mediation, Mendelian randomization analysis, psoriasis

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Introduction

Idiopathic thrombocytopenic purpura is an acquired autoimmune hemorrhagic disorder characterized by a reduction in platelet counts and an increased risk of bleeding.[1] The incidence of bleeding-induced hospitalization within 5 years after diagnosis is approximately 15%.[2] Traditional treatment relies on immunosuppression. Older immunosuppressants cannot change the natural course of the disease and affect the quality of life of patients. New therapies such as thrombopoietin receptor agonists can improve the quality of life of patients, but they are costly and there are issues of drug resistance.[3] Therefore, early prevention of ITP and exploration of new treatment regimens are needed.

To prevent the occurrence and progression of ITP, scholars have explored its risk factors. Previous studies have indicated that ITP is associated with a series of autoimmune diseases.[4-10] In observational studies, there are multiple confounding factors between autoimmune diseases and ITP, such as age, sex, viral infection, drugs, and the environment.[11] These factors may affect the correct judgment of the relationship between the two. Reverse causal bias may also mislead the understanding of the relationship between autoimmune diseases and ITP. More scientific research methods are needed to reduce its impact. To analyze the causal relationship between ITP and autoimmune diseases more effectively for prevention and management, we used MR to clarify this issue.

Idiopathic thrombocytopenic purpura is closely related to the occurrence and progression of multiple immune cells. In ITP, abnormal activation of B cells leads to the production of a large number of anti-platelet

antibodies, causing platelet destruction. The imbalance of T cell subsets results in the inability to effectively regulate the autoimmune response. In addition, macrophages mediate platelet destruction.[1] Therefore, this study also deeply explores the intermediary role of 731 immune cells in autoimmune diseases that mediate ITP, which is of great significance for formulating more effective treatment strategies and accurately assessing the disease condition.

Mendelian randomization employs genetic variations as instrumental variables (IVs) for the purpose of assessing the causal relationship between risk factors (exposures) and outcomes. Given that single nucleotide polymorphisms (SNPs) are randomly assigned at conception, MR is less prone to confounding, measurement error, and reverse causality than many other observational methods are.[12] Given that immune cells play an important role in autoimmune diseases, we also performed two-step MR to estimate the proportion of immune cells contributing to autoimmune disease-mediated ITP. The complexity of the MR framework can be understood from Figures 1 and 2.

Material and Methods

Source of Data

The datasets of ITP (ebi-a-GCST90018865), PsO (ukb-a-100), CD (finn-b-K11_KELACROHN), UC (finn-b-ULCERNAS), MS (ukb-17670), CeD (ebi-a-1058), RA (bbj-a-73), SLE (ebi-a-GCST003156), AIH (finn-b-AUTOIMMUNE_HYPERTHYROIDISM), AS (finn-b-M13_ANKYLOSPON_STRICT), and T1D (finn-b-E4_DM1) originate from the Open Genome-Wide Association Study (GWAS) database of the Integrative Epidemiology Unit (IEU) (https://gwas.mrcieu.ac.uk/). The ebi-a-GCST90018865 dataset encompasses 24 199 770 SNPs derived from 489 424 samples. The ukb-a-100 dataset comprises 10 894 596 SNPs from 337 159 samples. The finn-b-K11_KELACROHN dataset contains 16 380 466 SNPs from 218 792 samples. The finn-b-ULCERNAS dataset consists of 16 380 457 SNPs from 212 507 samples. The ukb-b-17670 dataset includes 9 851 867 SNPs from 462 933 samples. The ieu-a-1058 dataset consists of 38 037 SNPs from 24 269 samples. The bbj-a-73 dataset comprises 8 747 962 SNPs from 8383 samples. The ebi-a-GCST003156 dataset contains 7 071 163 SNPs from 14 267 samples. The

Main Points

- Bidirectional two-sample MR analysis revealed the causal associations between 10 autoimmune diseases and ITP.
- The causal effects of MS, CeD, SLE, and AIH on the risk of ITP have been substantiated.
- Rheumatoid arthritis may potentially be a risk factor for ITP.
- PsO, CD, and UC are protective factors for ITP.
- All results meet the requirements for heterogeneity and horizontal pleiotropy.
- There is insufficient evidence to establish a causal relationship between AS and T1DM and ITP. No significant reverse causal relationships were detected.
- The two-step MR approach revealed that immune cells act as mediators between CeD and ITP as well as between CD and ITP.

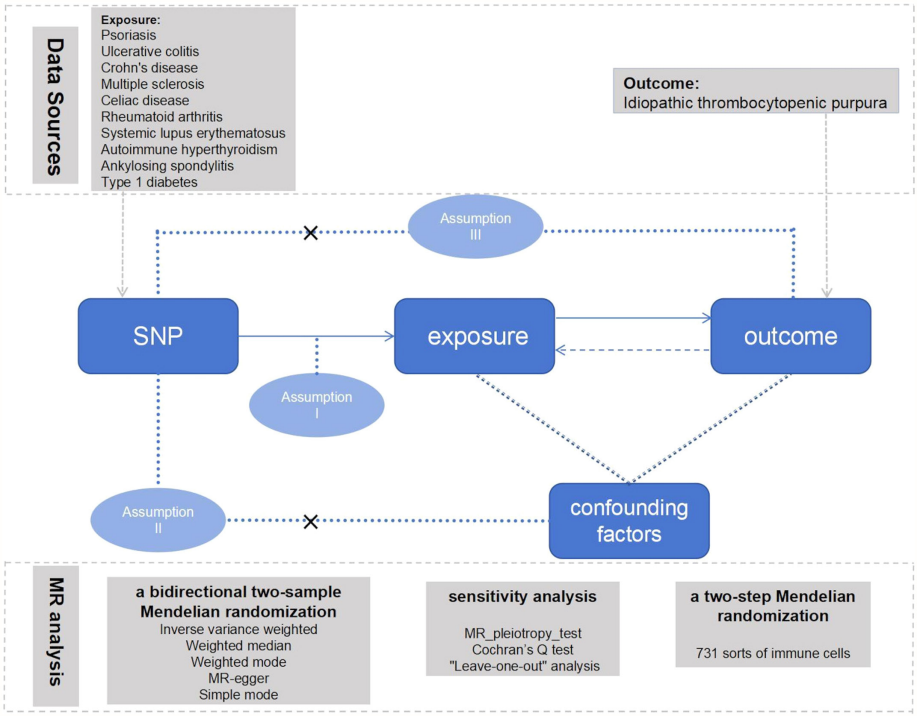

Figure 1. Overview of the research design of two-way two-sample MR. The MR framework is based on three fundamental MR assumptions. MR, Mendelian randomization; SNP, single nucleotide polymorphism.

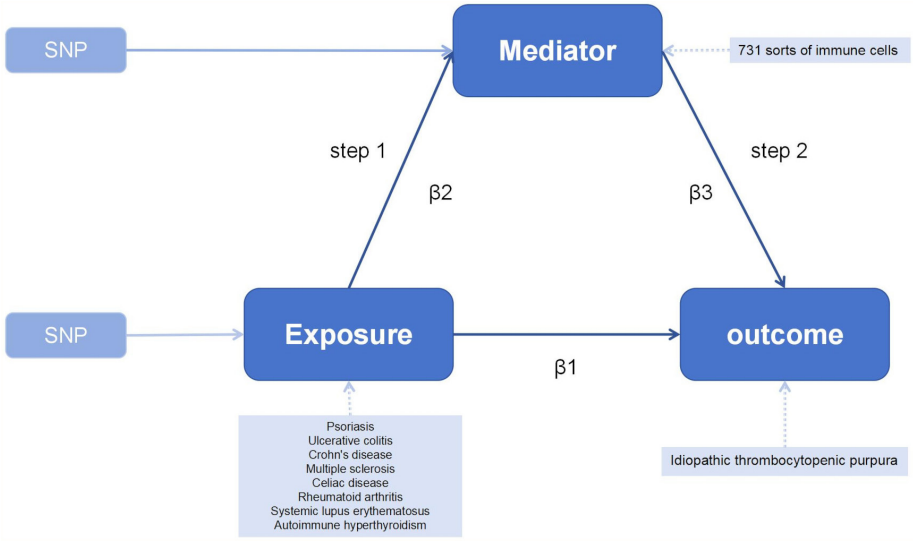

Figure 2. Overview of the two-step MR study design. Intermediate effect = β2 × β3. Intermediate ratio = β2 × β3/β1. Legend of scatter plot: Inverse variance weighted; MR Egger; Simple mode; Weighted median; Weighted mode. Legend of funnel plot: Inverse variance weighted; MR Egger.

fin-b-autoimmune_hyperthyroidism dataset includes 16 380 189 SNPs from 173 938 samples. The fin-b-m13_ankylospon_strict dataset contains 16 380 466 SNPs from 218 030 samples. The fin-b-e4_dm1 dataset comprises 16 380 008 SNPs from 189 113 samples. Details of the above datasets are given in Table 1. The data for MR analysis originated from the public genome-wide association studies (GWAS) database (https://gwas.mrcieu.ac.uk). In the original studies, relevant ethical approvals and informed consent of patients were obtained.

Genetic Instrument Selection

For forward two-sample MR analysis, the extract_instruments function of the TwoSampleMR software package was employed for the reading and filtering of exposure factors ($P < 5 \times 10^{-8}$).[13] In the reverse two-sample MR analysis, as an insufficient number of summary statistics for ITP could not be extracted, the threshold was adjusted to $P < 1 \times 10^{-5}$.[14] Linkage disequilibrium analysis (LDA) was used to eliminate single nucleotide polymorphisms (SNPs) ($r^2 = 0.001$, kb = 10 000), and all the F statistics were greater than 10 (Supplementary Table 1). When selecting exposed SNPs, we adopted three core assumptions: (1) genetic variations are closely associated with the exposure of interest; (2) they have no association with any known or potential confounding factors; and (3) except for the exposure, genetic variations have no relation to other pathways that affect the outcome and cannot be directly correlated with the outcome. Detailed SNP data can be found in Supplementary Table 1.

Bidirectional Two-Sample MR Analysis, Sensitivity Analysis and Visualization of Results

First, five distinct MR approaches, namely, MR–Egger,[15] weighted median,[16] inverse variance weighted (IVW),[17] simple mode, and weighted mode,[18] were utilized to investigate the causal associations between 10 autoimmune disorders and ITP, with IVW being of the utmost significance. In MR studies, OR is an important statistical measure used to quantify the strength of the association between exposure factors and outcomes, and it helps researchers infer causal relationships. An OR > 1 indicates that the exposure factor constitutes a risk factor, whereas an OR < 1 indicates that the exposure factor serves as a protective factor. The results are presented via scatter plots and forest plots in supplementary materials.

Furthermore, a sensitivity analysis was performed to evaluate the reliability of the MR findings. First, the horizontal pleiotropy test was executed in R via the TwoSampleMR function mr_pleiotropy_test. If $P > .05$, it implies the absence of horizontal pleiotropy, indicating that there are no confounding factors in this research.[17] Additionally, the Cochran's Q test was utilized for heterogeneity assessment. Specifically, in the Cochran's Q test, when the p value is greater than 0.05, heterogeneity is nonexistent. If heterogeneity existed ($P < .05$), the IVW test was adopted to test the random effects. In addition, a "Leave-one-out" analysis was conducted by progressively eliminating each SNP. If the impact of the remaining SNPs on the outcome variable does not undergo significant changes, this suggests that the MR analysis results are dependable. Next, we also conducted funnel plot analysis to detect potential publication bias. Finally, FDR is an important measure in Mendelian randomization studies as it helps control for the risk of false positives and provides a more accurate interpretation of the results. The p value of the univariate analysis results was corrected via the FDR method (a p value of FDR < 0.05 indicates statistical significance), which improved the reliability of the positive results. When P < FDR < 0.05, there is a significant association between exposure and outcome, which provides strong evidence for a possible causal relationship between exposure factors and outcomes. When P < 0.05 < FDR, there is a suggestive association between exposure and outcome. Although it does not reach the traditional statistical significance level, the result still has a certain suggestive effect, indicating a possible association.[19]

Mediation Analysis

Additionally, we employed a two-step MR study to explore whether the 731 kinds of immune cells mediate the causal routes of these eight diseases (PsO, CD, UC, MS, CeD, RA, SLE, and AIH) as exposures to ITP. The total effect was decomposed into the mediating effect and the direct effect. The formula for computing the direct effect is as follows: Direct effect = Total effect − Mediating effect.[20] The mediating roles of the exposure on ITP were subsequently disaggregated into (i) the causal effect of exposure to the mediator ($\beta 2$) and (ii) the causal effect of the mediator on the outcome ($\beta 3$). Mediation effect = $\beta 2 \times \beta 3$, Intermediate ratio = $\beta 2 \times \beta 3/\beta 1$.[20]

Results

Bidirectional Two-Sample MR Analysis

The IVW method shows significant associations between PsO and ITP ($\beta = -8.686$, se = 3.639, odds ratio (OR): 1.690E−04, 95% confidence interval (CI): 1.350E −07 to 0.211, $P = .017$, FDR = 0.42), between UC and ITP ($\beta = -0.344$, se = 0.149, OR: 0.709, 95% CI: 0.530 to 0.949, $P = .021$, FDR = 0.042), between CD and ITP

Table 1. Detailed Information About the Datasets

Trait	Year	Population	Author	GWAS ID	Sample Size	SNP
ITP	2021	European	Sakaue S	ebi-a-GCST90018865	489 424	24199770
PSO	2017	European	Neale	ukb-a-100	337 159	10894596
CD	2021	European	NA	finn-b-K11_KELACROHN	218 792	16380466
UC	2021	European	NA	finn-b-ULCERNAS	212 507	16380457
MS	2018	European	Ben Elsworth	ukb-b-17670	462 933	9851867
CeD	2011	European	Trynka	ieu-a-1058	24 269	38037
RA	2019	European	Ishigaki K	bbj-a-73	8383	8747962
SLE	2015	European	Bentham J	ebi-a-GCST003156	14 267	7071163
AIH	2021	European	NA	finn-b-AUTOIMMUNE_HYPERTHYROIDISM	173 938	16380189
AS	2021	European	NA	finn-b-M13 ANKYLOSPON_STRICT	218 030	16380466
T1DM	2021	European	NA	finn-b-E4_DM1	189 113	16380008

Table 2. Summary of inverse variance weighted results in forward two-sample MR analysis

outcome	exposure	β	se	or	or_lci95	or_uci95	pval	FDR
ITP	PSO	-8.686	3.639	1.690E-04	1.350E-07	0.211	0.017	0.042
ITP	UC	-0.344	0.149	0.709	0.530	0.949	0.021	0.042
ITP	CD	-0.203	0.099	0.816	0.673	0.990	0.039	0.049
ITP	CeD	0.159	0.056	1.173	1.050	1.310	0.005	0.023
ITP	SLE	0.066	0.031	1.068	1.005	1.136	0.034	0.049
ITP	MS	38.606	17.706	5.840E+16	49.513	6.890E+31	0.029	0.049
ITP	AIH	0.235	0.081	1.265	1.080	1.483	0.004	0.037
ITP	RA	0.106	0.044	1.112	1.020	1.214	0.017	0.055
ITP	AS	0.106	0.027	1.016	0.963	1.072	0.553	0.553
ITP	T1DM	0.035	0.054	1.035	0.931	1.151	0.519	0.577

(β = −0.203, se = 0.099, OR: 0.816, 95% CI: 0.673 to 0.990, *P* = 0.039, FDR = 0.049), between CeD and ITP (β = 0.159, se = 0.056, OR: 1.173, 95% CI: 1.050 to 1.310, *P* = .005, FDR = 0.023), between SLE and ITP (β = 0.066, se = 0.031, OR: 1.068, 95% CI: 1.005 to 1.136, *P* = .034, FDR = 0.049), between MS and ITP (β = 38.606, se = 17.706, OR: 5.840E + 16, 95% CI: 49.513 to 6.890E + 31, *P* = .029, FDR = 0.049), and between AIH and ITP respectively (β = 0.235, se = 0.081, OR: 1.265, 95% CI: 1.080 to 1.483, p = 0.004, FDR = 0.037). The IVW method shows a suggestive association between RA and ITP (β = 0.106, se = 0.044, OR: 1.112, CI: 1.020 to 1.214, *P* = .017, FDR = 0.055). The IVW method shows that there is currently insufficient evidence to prove a causal relationship between AS (β = 0.016, se = 0.027, OR: 1.016, 95% CI: 0.963 to 1.072, *P* = .553, FDR = 0.553) and T1DM (β = 0.035, se = 0.054, OR: 1.035, 95% CI: 0.931 to 1.151, *P* = .519, FDR = 0.577) and ITP. Refer to Supplementary Figures 1-16 for visualization of the results (forest plots, scatter plots). For forward MR analysis, the results of methods such as MR–Egger, weighted median, simple mode, and weighted mode are presented in detail in Table 2 Supplementary Table 2. In reverse MR analysis, there is insufficient evidence to prove a reverse causal relationship between these ten autoimmune diseases and ITP. See Supplementary Table 3. (Insufficient summary statistics of CeD could not be extracted for MR analysis.)

Sensitivity Analyses

A comprehensive sensitivity analysis is performed to assess the reliability of the causal relationship obtained due to violation of the IV hypothesis. First, via MR–Egger regression intercept analysis, there was no evidence of horizontal pleiotropy among IVs. The *P* value of all associated MR–Egger intercepts was >.05 (Supplementary Table 4). Second, the *P* value (>.05) of Cochran's Q test basically excludes heterogeneity (Supplementary Table 5). Next, the results of the leave-one-out method further verify the persistence of the results (leave-one-out analysis plots in Supplementary Figures 17-24). Finally, The funnel plot shows good symmetry (funnel plots in Supplementary Figures 25-32). In conclusion, the stability of the results is supported by strict sensitivity analysis.

A Two-Step MR

The Immune Effector Mechanism in ITP

All the diseases were subjected to reverse MR studies, and no significant causal relationships were found. This ensures that the mediation pathway can proceed only in the direction of exposure → mediator → outcome. It is known that immune cells play important roles in the pathogenesis of autoimmune diseases. Therefore,we explored the relationship between 731 types of immune cells and ITP to identify potential mediators in the pathway linking exposure and outcome. The data of 731 types of immune cells can be openly obtained from the GWAS Catalogue (https://www.ebi.ac.uk/gwas/, accession numbers from GCST90001391 to GCST90002121). The reading and filtering of the exposure factors are performed via the extract_instruments function of the TwoSampleMR software package ($P < 5 \times 10^{-8}$). Instrumental variables are removed via LDA ($r^2 = 0.001$, kb = 10 000) and comply with three core assumptions. IVW MR analysis revealed significant correlations: (1) Switched memory B cell %lymphocyte is associated with ITP (β = 0.503, se = 0.242, OR = 1.653, 95% CI 1.029 to 2.656, *P* = .038, FDR = 0.044); (2) B cell %CD3− lymphocyte is associated with ITP (β = −0.170, se = 0.073, OR= 0.843, 95% CI 0.730 to 0.974, *P* = .020, FDR = 0.029); (3) CD20 on IgD+ CD38+ B cell is associated with ITP (β = −0.420, se = 0.123, OR = 0.657, 95% CI 0.516-0.837, *P* = .001, FDR = 0.002); (4) CD20 on IgD+ CD24− B cell is associated with ITP (β = −0.559,

Table 3. Summary of Inverse Variance Weighted Results from the MR Analysis of Immune Cells and ITP

outcome	exposure	β	se	or	or_lci95	or_uci95	pval	FDR
ITP	Switched memory B cell %lymphocyte	0.503	0.242	1.653	1.029	2.656	0.038	0.044
ITP	B cell %CD3-lymphocyte	-0.170	0.073	0.843	0.730	0.974	0.020	0.029
ITP	CD20 on IgD+CD38+B cell	-0.420	0.123	0.657	0.516	0.837	0.001	0.002
ITP	CD20 on IgD+CD24-B cell	-0.559	0.119	0.572	0.452	0.723	2.880E-06	2.016E-05
ITP	CD20 on naive-mature B cell	-0.427	0.136	0.653	0.500	0.851	0.002	0.004
ITP	CD20 on IgD+B cell	-0.404	0.129	0.667	0.518	0.860	0.002	0.003
ITP	CD25 on IgD+CD38+B cell	0.572	0.288	1.771	1.008	3.113	0.047	0.047

Table 4. Summary of Inverse Variance Weighted Results in MR Analysis of Exposures and Mediators

outcome	exposure	β	se	or	or_lci95	or_uci95	pval	FDR
CD20 on IgD+CD24-B cell	CD	0.106	0.041	1.112	1.027	1.205	0.009	0.018
CD20 on IgD+B cell	CD	0.101	0.040	1.106	1.022	1.198	0.012	0.012
CD20 on IgD+CD24-B cell	CeD	-0.121	0.031	0.886	0.833	0.942	1.018E-04	1.357E-04
CD20 on IgD+CD38+B cell	CeD	-0.131	0.031	0.877	0.825	0.932	2.459E-05	9.835E-05
CD20 on naive-mature B cell	CeD	-0.128	0.031	0.880	0.828	0.935	4.106E-05	8.212E-05
CD20 on IgD+B cell	CeD	-0.121	0.031	0.886	0.834	0.942	1.152E-04	1.152E-04

se=0.119, OR=0.572, 95% CI 0.452 to 0.723, *P*=2.880E–06, FDR=2.016E–05); (5) CD20 on naive-mature B cell is associated with ITP (β=–0.427, se=0.136, OR=0.653, 95% CI 0.500 to 0.851, *P*=0.002, FDR=0.004); (6) CD20 on IgD+ B cell is associated with ITP (β=–0.404, se=0.129, OR=0.667, 95% CI 0.518 to 0.860, *P*=.002, FDR=0.003); (7) CD25 on IgD+ CD38+ B cell is associated with ITP (β=0.572, se=0.288, OR=1.771, 95% CI 1.008 to 3.113, *P*=.047, FDR=0.047). The results mainly refer to the IVW method. The results refer mainly to the IVW method. For the results of the other methods, Table 3 see Supplementary Table 6 for details. No significant causal relationships were found between other immune cells and ITP. No evidence of horizontal pleiotropy and heterogeneity was found (Supplementary Tables 7 and 8). See Supplementary Table 9 for the F statistics and detailed SNPs.

Subsequently, the relationship between Switched memory B cell %lymphocyte, B cell %CD3 - lymphocyte, CD20 on IgD+ CD38+ B cell, CD20 on IgD+ CD24– B cell, CD20 on naive - mature B cell, CD20 on IgD+ B cell, CD25 on IgD+ CD38+ B cell and PsO, CD, UC, CeD, RA, MS, SLE, and AIH was studied. Among the studied components, the IVW method revealed that CeD was associated with CD20 on IgD+ CD38+ B cell (β=–0.131, se=0.031, OR=0.877, 95% CI 0.825 to 0.932, p=2.459E05, FDR=9.835E–05); CeD was associated with CD20 on IgD+ CD24– B cell (β=–0.121, se=0.031, OR=0.886, 95% CI 0.833 to 0.942, *P*=1.018E–04, FDR=1.357E–04); CeD was associated with CD20 on IgD+ B cell (β=–0.121, se=0.031, OR=0.886, 95% CI 0.834 to 0.942, p=1.152E–04, FDR=1.152E–04); and CeD was associated with CD20 on naive-mature B cell (β=–0.128, se=0.031, OR=0.880, 95% CI 0.828 to 0.935, *P*=4.106E–05, FDR=8.212E05). CD was associated with CD20 on IgD+ B cell (β=0.101, se=0.040, OR=1.106, 95% CI 1.022 to 1.198, *P*=.012, FDR=0.012), CD was associated with CD20 on IgD+ CD24- B cell (β=0.106, se=0.041, OR=1.112, 95% CI 1.027-1.205, *P*=0.009, FDR=0.018). See Supplementary Table 10 for the F statistics and detailed SNPs. (The results of MR–Egger, weighted median, simple model and weighted model are shown in Table 4 Supplementary Table 11).

Mediator analysis revealed that CD20 on IgD+ CD38+ B cell played a partial mediating role in the causal pathway from CeD to ITP. The mediating effect of the delta method was 0.055 (se: 0.021, 95% CI 0.019-0.102, or: 1.057, or_lci95: 1.019, or_uci95: 1.107), and the proportion of the mediating effect was 34.50%. CD20 on IgD+ CD24- B cell plays a partial mediating role in the causal pathway from CeD to ITP. The mediating effect of the delta method was 0.056 (se: 0.022, 95% CI 0.019-0.105, or: 1.058, or_lci95: 1.019, or_uci95: 1.111), and the proportion of the mediating effect was 35.13%. CD20 on IgD+ B cell plays a partial mediating role in the causal pathway from CeD to ITP. The mediating effect of the delta method was 0.035 (se: 0.02, 95% CI 0.001 to 0.079, or: 1.036, or_lci95: 1.001, or_uci95: 1.082), and the proportion of the mediating effect was 21.96%. CD20 on naive-mature B cell partially mediated the causal pathway from CeD to ITP. The mediating effect of the delta method was 0.039 (se: 0.022, 95% CI 0.001-0.087, or: 1.039, or_lci95: 1.001, or_uci95: 1.091), and the percentage of the mediating effect was 24.46%. CD20 on IgD+ B cell plays a partial mediating role in the causal pathway from CD to ITP. The mediating effect of the delta method was –0.041 (se: 0.022, 95% CI 0.09-0.006, or: 0.96, or_lci95: 0.914, or_uci95: 0.994), and the proportion of the mediating effect was 20.19%. CD20 on IgD+ CD24- B cell plays a partial mediating role in the causal pathway from CD to ITP. The mediating effect of the delta method was –0.059 (se: 0.027, 95% CI 0.117-0.013, or: 0.942, or_lci95: 0.89, or_uci95: 0.987), and the percentage of the mediating effect was 29.05%.

Discussion

Exploring risk factors for ITP is crucial. This study establishes causal links between certain autoimmune diseases and ITP. Rheumatoid arthritis shows no correlation. MS, CeD, SLE, and AIH affect ITP risk. PsO, CD, and UC are protective. CD20 on specific B cells mediates associations between CeD and ITP and between CD and ITP.

Studies show an increased platelet count in psoriasis patients. IL-23 binds to receptors, activating JAK2 and leading to psoriasis.[21] JAK2 is activated with the thrombopoietin receptor, promoting platelet production.[22] In this context, a scientific hypothesis was put forward: The JAK-STAT signaling pathway plays a crucial role in regulating the physiological state of platelets in patients with PSO, and this regulation may be one of the reasons why PSO patients are less likely to suffer from ITP. It should be emphasized, however, that at present this is merely a speculation based on the existing research data. Although these findings provide a new potential direction for the research on the treatment of ITP, it must recognize that the mechanisms of reactive thrombocytosis caused by inflammation may be complex and diverse in different diseases. Therefore, more in-depth studies are needed to comprehensively analyze the differences of this potential mechanism in different diseases. Studies have shown that the platelet counts in patients with UC and CD are increased, and the levels of thrombopoietin in patients with inflammatory bowel disease (IBD) are higher than those in the general population, which may be the reason why patients with UC and CD are less likely to suffer from ITP.[5,23] Remarkably, an MR study conducted by Li H et al[24] demonstrated that genetically predicted UC and CD are both positively correlated with ITP. The likely reason for this disparity may be that geography and ethnicity can modify causal relationships. In recent years, the relationship between ITP and CeD has attracted increasing attention.[25] Combined with the results of MR, it is reasonable to believe that patients with CeD may develop ITP. Therefore, patients with CeD should be vigilant about the occurrence and progression of ITP. For celiac patients with ITP, a gluten-free diet can be used to treat the symptom of decreased platelet count. This can not only ensure the life safety of patients and improve their quality of

life, but also reduce family economic burdens and social fiscal expenditures. This will be of great significance for clinicians. Several studies from different countries have evaluated the causal relationship between ITP and SLE, but no consensus has been reached yet.[6,7] From the perspective of MR studies, it has been confirmed that SLE is a risk factor for ITP and there is no reverse causal relationship. Studies have shown that the prevalence of MS in patients with ITP is 25 times higher than that expected in the general population.[9] However, the exact connection between the coexistence of the two has not been fully explained. MS has been identified as a potential cause of ITP in our study. Nevertheless, reports on MS combined with ITP in the literature are limited. Future clinical studies with larger sample sizes are still needed to confirm these findings and explore the underlying mechanisms. Study have found that ITP can coexist with AIH, and platelet-associated IgG and/or specific circulating platelet autoantibodies can be detected in patients with AIH. Treating underlying thyroid diseases may improve thrombocytopenia.[10] The authors have confirmed the unidirectional causal relationship between AIH and ITP. It is recommended to perform routine assessment of thyroid function and actively treat thyroid diseases in patients with ITP.[10] MR analysis results suggest that RA may be a risk factor for ITP. In clinical settings, thrombocytopenia in patients with RA is a rare complication. It may be that the frequent use of corticosteroid treatment in these patients masks the association with thrombocytopenia. The relationship between RA and ITP is uncertain and requires further exploration.

In ITP, the autoantibodies against self-antigens produced by autoreactive B cells, especially the autoantibodies against glycoprotein IIb (GPIIb)/IIIa and/or GPIb/IX, that is, the loss of immune tolerance to platelet self-antigens, constitute a key upstream step in the pathophysiology of ITP. In addition, abnormalities in B-cell subsets, such as defective regulatory B cells (Bregs), the expansion of memory B cells, and long-lived plasma cells, play a crucial role in the production of autoantibodies in ITP.[1] Studies have found that B cells play a role in the pathogenesis of celiac disease by promoting the cytotoxic potential of intraepithelial lymphocytes (IELs) and the development of villous atrophy (VA).[26]

Although these studies highlight the important role of immune cells and their complex phenotypes in disease development, the results may be influenced by potential confounding factors and limited sample sizes. Therefore, we designed a two-step MR study to investigate the mediating role of 731 immune cell phenotypes, aiming to provide new insights into the pathogenesis and treatment methods of ITP. The results of the two-step MR study revealed that the association between CD and ITP is mediated by disorders of CD20 on IgD+ CD24- B cell and CD20 on IgD+ B cell; the association between CeD and ITP is mediated by disorders of CD20 on IgD+ CD38+ B cell, CD20 on IgD+ CD24- B cell, CD20 on IgD+ B cell, and CD20 on naive-mature B cell.

CD20 plays a crucial role in the activation, proliferation and differentiation processes of B cells.[27] B lymphocytes can destroy platelets by generating autoantibodies against platelets.[1] Clinically, anti-CD20 monoclonal antibodies can bind to CD20 on the surface of B cells, inducing apoptosis of B cells, thereby alleviating the destruction of platelets.[28] In healthy people, B cells switching to the IgD isotype are mainly autoreactive. A small portion of B cells form Cδ-cs cells through conversion in a specific switch region.[29] Human Cδ-CS B lymphocytes are autoreactive, and their generation may be related to immune tolerance mechanisms.[30] CD38 participates in the regulation of autoimmunity. Current studies have confirmed that the elimination of CD38 by anti-CD38 monoclonal antibodies can downregulate the production of autoantibodies and restore immune balance by reducing platelet destruction and increasing platelet count.[31]

Although this study has alleviated some of the limitations of traditional observational studies, there are still some deficiencies and prospects for the future. First, only the causal relationship between autoimmune diseases and ITP has been established. The specific mechanism and the causal relationship with disease severity and duration need further study. Second, the GWAS data used in this study is from the European population, which may limit the generalizability of this research results in other ethnic or geographical groups. With the continuous improvement of databases, it is expected that these findings can be confirmed in different populations in the future. Sensitivity analysis of a more extensive sample will also strengthen the results. Third, in this study, the MR-egger intercept analysis was utilized to examine horizontal pleiotropy. No significant impact of horizontal pleiotropy was observed. However, it is possible that the pleiotropic functions of certain genes have yet to be discovered. It is looked forward to that additional pleiotropy control methods will be explored in future studies. Fourthly, due to the limitations of data sources, subgroup analysis and more comprehensive analysis of autoimmune diseases cannot be performed. This gap needs to be filled by the continuous improvement of future databases. The authors hope that researchers around the world consider these preliminary results and conduct multicenter clinical cohort studies to further understand the causal relationships between MS, CD, RA, SLE, AIH, PsO and ITP, as well as the mediating role of immune cells. Fifthly, sensitivity analyses of horizontal pleiotropy, heterogeneity, and the leave-one-out sensitivity analysis were performed in the study. Future research could be committed to employing more comprehensive sensitivity analysis methods and exploring and developing new methods for comprehensively evaluating and correcting various biases that might influence the research results. Finally, this study has revealed the mediating roles of certain immune cells. However, in the complex context of autoimmune diseases, there is a significant scarcity of research literature regarding the specific mechanisms by which these immune cells affect ITP. This situation suggests that in-depth exploration around this aspect can be carried out to fill the knowledge gap in this field.

Conclusion

In summary, through MR analysis, the authors have revealed the causal relationship between ten autoimmune diseases and ITP, and determined the causal relationship between eight autoimmune diseases and ITP. Therefore, it is of great significance to regularly monitor the blood routine of patients with MS, CD, RA, SLE, and AIH and take preventive measures. It also emphasizes the potential of a gluten-free diet for the treatment of ITP. At the same time, PsO, CD, and UC as protective factors for ITP, as well as the mediating role of immune cells, provide ideas and evidence for actively seeking new drug targets.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

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