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ORIGINAL ARTICLE

Investigation of the Influence of Immune Cells on Aplastic Anemia: A Study Based on Genome-Wide Association Studies Data

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Abstract:
Aplastic anemia (AA) is a non-neoplastic bone marrow failure syndrome characterized by the destruction of hematopoietic stem and progenitor cells, leading to a reduction in the production of all types of blood cells. This destruction is often caused by an abnormal immune response. This study aims to explore the association between 731 immunophenotypes and genetic susceptibility to AA. One AA-related GWAS dataset (ebi-a-GCST90018794) was obtained from the IEU OpenGWAS project. The ebi-a-GCST90018794 performed a GWAS on 473,500 European individuals with approximately 24,192,378 SNPs analyzed after quality control and imputation. GWAS summary statistics for each immune cell are publicly available from the European Bioinformatics Institute (accession numbers from GCST0001391 to GCST0002121). A total of 731 immunophenotypes were included. The Mendelian randomization analysis reported that 5 types of immune cells were associated with increased genetic susceptibility to AA. Inverse variance weighting (IVW) showed that CD127- CD8br T cell [odds ratio (OR), 1.135; 95% confidence interval (CI), 1.032–1.247; p = 0.009], CD25 on IgD+ CD38dim (OR, 1.053; 95% CI, 1.013–1.095; p = 0.0091), CD39+ resting Treg CD4 Treg (OR, 1.034; 95% CI, 1.010–1.059; p = 0.005), CD39+ secreting Treg AC (OR, 1.050; 95% CI, 1.013–1.089; p = 0.007), and CD8 on CD28+ CD45RA- CD8br (OR, 1.127; 95% CI, 1.044–1.215; p = 0.002) were associated with increased genetic susceptibility to AA. Conversely, 4 types of immune cells were associated with decreased genetic susceptibility to AA, including CD86 on CD62L+ myeloid DC [odds ratio (OR), 0.930; 95% confidence interval (CI), 0.882–0.981; p = 0.008], CD86+ plasmacytoid DC AC (OR, 0.923; 95% CI, 0.857–0.994; p = 0.034), DC AC (OR, 0.897; 95% CI, 0.838–0.960; p = 0.002), and DN (CD4-CD8-) NKT lymphocyte (OR, 0.926; 95% CI, 0.869–0.986; p = 0.017). This study explored the association between 731 immunophenotypes and genetic susceptibility to AA, identifying multiple immune cells associated with genetic susceptibility to AA. This provides a new perspective for the study of the pathogenesis of AA.

Key Words: Aplastic anemia, Mendelian randomization, Genetic susceptibility, Immune cells, GWAS

Introduction

Aplastic anemia (AA) is a severe bone marrow failure syndrome characterized by decreased blood cell production due to the destruction of hematopoietic stem cells and precursor cells in the bone marrow [1]. This condition typically presents with anemia, a tendency to bleed, and an increased susceptibility to infections. AA can occur at any age but is more common in young and middle-aged individuals [2]. Although it is a rare disease, AA has been reported worldwide. Its pathogenesis involves multiple factors, including the immune system attacking bone marrow cells, genetic factors, environmental exposures, and drug exposures [3-4]. Treatment methods for AA include blood transfusions, immunosuppressive therapy, and hematopoietic stem cell transplantation [5-6]. However, some patients may require a suitable hematopoietic stem cell donor for transplantation. Despite progress in treatment, AA continues to significantly impact the lives and

health of patients.

The pathogenesis of AA is complex, involving defects in hematopoietic stem cells, abnormalities in the bone marrow microenvironment, immune dysfunction, and somatic mutations, with immune mechanisms playing a critical role [7-10]. Immune cells are closely linked to the development of AA [11-12]. In AA, the abnormal activation and dysfunction of immune cells can lead to attacks on hematopoietic stem cells and the bone marrow microenvironment, resulting in bone marrow failure [13]. T lymphocytes, in particular, play a significant role in the pathogenesis of AA. Research suggests that in some AA patients, the function of T lymphocytes is abnormal, which may lead to attacks on hematopoietic stem cells and the bone marrow microenvironment. This can involve abnormal activation of immune cells mediated by T cells or impaired function of immunoregulatory cells [14]. In addition to T cells, NK cells (natural killer cells) are also believed to contribute to the pathogenesis of AA. Studies have shown that NK cells may have direct cytotoxic effects on hematopoietic stem cells, causing bone marrow failure [15]. Furthermore, cytokines released by immune cells, such as interferon-gamma (IFN-γ) and tumor necrosis factor (TNF), may also negatively impact hematopoietic stem cells and the bone marrow microenvironment [16-17]. Overall, the abnormal activation and dysfunction of immune cells are significant factors in the pathogenesis of AA. In-depth research into these mechanisms can improve the understanding of AA and provide a theoretical basis for developing new therapeutic strategies.

Mendelian randomization (MR) is a technique that utilizes genetic variants for causal inference and is extensively employed in medical research. This method capitalizes on the random influence of genetic variants on a biological trait or disease risk, thereby assisting researchers in deducing causal relationships. In medical research, Mendelian randomization can be employed to address the frequent issue of confounding in observational studies and to infer causal relationships rather than mere correlations. By using genetic variants that affect a particular biological trait or disease, researchers can apply Mendelian randomization to evaluate the impact of an exposure on disease risk or biological traits, thus drawing conclusions about causal relationships [18-19]. In summary, the use of Mendelian randomization in medical research can enable researchers to more accurately assess the effects of exposure factors on disease risk or biological traits, thereby providing significant evidence to support the development of more effective prevention and treatment strategies.

Materials and Methods

Study Design

We employed MR to examine the causal relationship between 731 types of immune cells and AA. The instrumental variables in causal inference had to meet three essential conditions: (1) genetic variations must be directly related to exposure factors; (2) genetic variations must be unrelated to potential confounding factors between exposure and outcome variables; (3) genetic variations must not affect outcome variables through pathways other than exposure.

Genome-Wide Association Study (GWAS) Data Sources for AA

Genome-wide association analysis involves identifying sequence variations, known as single nucleotide polymorphisms (SNPs), within the human genome and selecting SNPs associated with diseases. An AA-related GWAS dataset (ebi-a-GCST90018794) was sourced from the IEU OpenGWAS project [20]. The ebi-a-GCST90018794 conducted a GWAS on 473,500 European individuals (Ncase = 4,128, Ncontrol = 469,372), with approximately 24,192,378 SNPs analyzed following quality control and imputation.

Immunity Cells Related GWAS Data Sources

GWAS summary statistics for each immune cell are publicly available from the European Bioinformatics Institute (www.ebi.ac.uk/) [21] (accession numbers from GCST0001391 to GCST0002121). A total of 731 immunophenotypes were included, encompassing monocytes, myeloid cells, T cells, B cells, natural killer cells, and others.

Selection of Instrumental Variables (IVs)

To ensure the comprehensiveness of the results, SNPs closely associated with specific immune cell phenotypes ($p < 1.0\times10\text{-}5$) were chosen as instrumental variables [22]. Linkage disequilibrium

analysis was conducted using European genomic sample data with parameter settings of r2 < 0.001 and kb = 10000. For AA data, the significance level was adjusted to P < 1×10-8 in the study. The F-statistic for each IV was calculated to assess the strength of instrumental variables and avoid weak instrument bias. An F-statistic greater than 10 indicated that the instrumental variables were not subject to weak instrument bias. The F-statistic was calculated using the formula: F = [R2/(1 − R2)]*[(N – K − 1)/K]. Here, K represented the number of paired samples, N denoted the total sample size, and R2 signified the cumulative explained variance [23].

Statistical Analysis

All analyses were performed using R 4.3.2 software (http://www.Rproject.org). To evaluate the causal association between 731 immunophenotypes and AA, inverse variance weighting (IVW) [24] and weighted median-based [25] methods were primarily used via the 'TwoSampleMR' package (version 0.6.3), 'VariantAnnotation' package, and 'gwasglue' package. Cochran's Q and leave-one-out methods were applied to analyze heterogeneity and sensitivity, respectively. When $p \geq 0.05$, there was no heterogeneity in the results.

Results

Two-sample MR Analysis

In our study, we intersected the results obtained from inverse variance weighting (IVW) and weighted median-based methods. The MR analysis identified five types of immune cells associated with an increased genetic susceptibility to AA (**Supplementary Table S1**).

Supplementary Table S1.1 Information of identified SNPs in exposure (CD127- CD8br T cell) and outcomes (AA).

				Exposure (CD127- CD8br T cell)			Outcome (AA)				
	SNP	EA	OA	β	SE	*p* value	Case	Control	β	SE	*p* value
1	rs10171072	A	G	0.1532	0.03199	1.75E-06	4,128	469,372	0.0043	0.0227	0.8502
2	rs1031000	T	C	0.1252	0.02768	6.31E-06	4,128	469,372	0.0137	0.0228	0.5482
3	rs117509659	C	T	0.8353	0.1843	6.06E-06	4,128	469,372	0.192	0.0927	0.0382499
4	rs13187738	G	A	-0.1922	0.03986	1.50E-06	4,128	469,372	-0.0164	0.0333	0.621101
5	rs143237141	A	G	0.3035	0.06717	6.45E-06	4,128	469,372	0.0523	0.0538	0.3311
6	rs143747981	G	A	-0.406	0.09157	9.56E-06	4,128	469,372	-0.0724	0.0403	0.0722104
7	rs144005685	C	T	-0.3525	0.07719	5.12E-06	4,128	469,372	-0.0567	0.1129	0.615601
8	rs144285452	G	C	-0.2353	0.05177	5.68E-06	4,128	469,372	0.1031	0.0992	0.2987
9	rs147338725	A	G	0.248	0.05471	6.05E-06	4,128	469,372	0.1582	0.1291	0.2204
10	rs176187	A	G	0.1931	0.0435	9.30E-06	4,128	469,372	0.0248	0.0357	0.4867
11	rs17792777	T	C	0.1255	0.0253	7.36E-07	4,128	469,372	-0.0643	0.0272	0.0181798
12	rs4920093	G	A	0.2628	0.05915	9.15E-06	4,128	469,372	-0.0315	0.0799	0.693499
13	rs6904669	G	A	-0.1557	0.02909	9.18E-08	4,128	469,372	-0.0539	0.0242	0.0259998
14	rs77729615	T	C	0.6817	0.1489	4.83E-06	4,128	469,372	0.2279	0.333	0.4938
15	rs915895	C	T	0.1817	0.03425	1.20E-07	4,128	469,372	0.0162	0.0271	0.5496

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; SE, standard error; AA, aplastic anemia.

Supplementary Table S1.2 Information of identified SNPs in exposure (CD25 on IgD+ CD38dim) and outcomes (AA).

				Exposure (CD25 on IgD+ CD38dim)			Outcome (AA)				
	SNP	EA	OA	β	SE	*p* value	Case	Control	β	SE	*p* value
1	rs11051398	T	C	-0.123	0.02607	2.48897E-06	4,128	469,372	0.0395	0.0635	0.5334
2	rs111741355	A	G	-2.854	0.5728	6.54892E-07	4,128	469,372	-0.1832	0.0975	0.0602601

3	rs112043339	T	C	-0.4991	0.1126	9.55608E-06	4,128	469,372	0.0132	0.1127	0.9065
4	rs11615354	T	C	0.19	0.0403	2.49799E-06	4,128	469,372	-0.0386	0.0811	0.634
5	rs116525939	C	T	0.954	0.2076	0.000004469	4,128	469,372	0.5453	0.3934	0.1657
6	rs116970311	A	C	-1.057	0.235	7.06806E-06	4,128	469,372	-0.0278	0.0953	0.7703
7	rs117156856	A	C	1.252	0.268	3.09999E-06	4,128	469,372	0.1053	0.0929	0.2571
8	rs117382470	T	C	1.143	0.253	6.47993E-06	4,128	469,372	0.0195	0.0518	0.706
9	rs144941792	T	G	0.2117	0.04191	4.61902E-07	4,128	469,372	-0.0964	0.0722	0.182
10	rs146720368	T	C	0.9178	0.1934	2.15001E-06	4,128	469,372	0.0813	0.0501	0.1046
11	rs147487472	A	C	0.4191	0.06162	1.19812E-11	4,128	469,372	0.0133	0.0406	0.7428
12	rs149662107	A	G	-0.9269	0.1592	6.267E-09	4,128	469,372	-0.1623	0.1531	0.289
13	rs34351616	G	A	-0.2632	0.05587	2.54302E-06	4,128	469,372	0.0252	0.0353	0.475
14	rs35704968	C	T	-0.353	0.07958	9.46194E-06	4,128	469,372	0.0787	0.0607	0.1946
15	rs3793662	T	C	-0.1575	0.02958	1.07399E-07	4,128	469,372	-0.0065	0.0272	0.8096
16	rs4869953	G	A	-0.1265	0.02541	6.69607E-07	4,128	469,372	-0.03	0.0227	0.1859
17	rs59757547	C	T	-0.1149	0.02559	7.36105E-06	4,128	469,372	-0.0097	0.0225	0.6674
18	rs62206862	G	C	-1.161	0.2449	2.22101E-06	4,128	469,372	-0.0376	0.2618	0.8859
19	rs62626323	A	C	0.5433	0.04021	1.25285E-40	4,128	469,372	0.0327	0.0593	0.581201
20	rs709589	T	C	-0.1507	0.03159	1.90301E-06	4,128	469,372	0.0063	0.0265	0.8121
21	rs72944931	G	A	0.4742	0.1056	7.33398E-06	4,128	469,372	0.0582	0.1099	0.596499
22	rs76687530	T	C	-0.194	0.04068	1.93798E-06	4,128	469,372	-0.0427	0.0348	0.2191
23	rs8028696	T	C	0.131	0.02766	2.26501E-06	4,128	469,372	6.00E-04	0.0264	0.9813
24	rs9425892	C	T	-0.2829	0.05977	2.29097E-06	4,128	469,372	-0.0867	0.0823	0.292

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; SE, standard error; AA, aplastic anemia.

Supplementary Table S1.3 Information of identified SNPs in exposure (CD39+ resting Treg CD4 Treg) and outcomes (AA).

				Exposure (CD39+ resting Treg CD4 Treg)			Outcome (AA)				
	SNP	**EA**	**OA**	**β**	**SE**	***p* value**	**Case**	**Control**	**β**	**SE**	***p* value**
1	rs10086961	C	T	-0.1275	0.02736	3.29602E-06	4,128	469,372	-0.0027	0.0227	0.9051
2	rs111534725	T	G	0.8056	0.06291	1.05512E-36	4,128	469,372	0.0115	0.1378	0.9337
3	rs113264169	T	C	0.2772	0.06262	9.87006E-06	4,128	469,372	-0.0274	0.1189	0.8176
4	rs113435341	G	A	2.267	0.2171	3.79315E-25	4,128	469,372	-0.0296	0.1384	0.8309
5	rs11579717	A	G	1.718	0.1835	1.35114E-20	4,128	469,372	0.0701	0.0441	0.112
6	rs115977640	A	C	0.6189	0.1337	3.82904E-06	4,128	469,372	-0.0301	0.0594	0.611799
7	rs116794175	C	T	1.311	0.1905	6.98232E-12	4,128	469,372	0.0933	0.2797	0.7387
8	rs116988060	T	A	1.095	0.2254	1.24799E-06	4,128	469,372	0.0507	0.0763	0.5063
9	rs117572834	G	T	-1.108	0.2135	2.25299E-07	4,128	469,372	-0.0626	0.0839	0.4555
10	rs11818051	T	C	0.6998	0.0719	4.23741E-22	4,128	469,372	0.3148	0.0993	0.001525
11	rs11890666	A	G	-0.1612	0.02787	7.94292E-09	4,128	469,372	9.00E-04	0.0298	0.9749
12	rs12258674	T	A	-0.1514	0.03401	8.80704E-06	4,128	469,372	-0.0693	0.0346	0.0452699
13	rs12518588	G	T	0.1241	0.02523	9.15398E-07	4,128	469,372	0.0066	0.0225	0.7701
14	rs12712610	G	A	0.201	0.02719	1.82684E-13	4,128	469,372	-0.0412	0.033	0.2126
15	rs12778837	C	T	0.1965	0.02651	1.5531E-13	4,128	469,372	-0.0297	0.0234	0.2055
16	rs1410600	T	C	-0.2216	0.02536	3.58922E-18	4,128	469,372	-0.0311	0.0226	0.1681
17	rs150956189	C	T	2.837	0.6244	5.74196E-06	4,128	469,372	0.0515	0.0596	0.3875

18	rs17111341	C	T	0.6853	0.05013	1.85909E-41	4,128	469,372	0.1537	0.0777	0.0477595
19	rs182702572	A	C	0.5464	0.07402	1.95119E-13	4,128	469,372	-0.05	0.068	0.4618
20	rs187089343	C	A	0.8819	0.1864	2.31702E-06	4,128	469,372	-0.1284	0.1011	0.2041
21	rs2326054	C	T	-0.2311	0.04963	3.34503E-06	4,128	469,372	0.0694	0.0904	0.4429
22	rs34645541	A	C	0.7843	0.1289	1.293E-09	4,128	469,372	0.0507	0.0805	0.5291
23	rs357363	G	C	0.3075	0.06798	6.32106E-06	4,128	469,372	-0.0211	0.0518	0.684301
24	rs35909109	T	C	0.3811	0.05993	2.29901E-10	4,128	469,372	0.0503	0.0577	0.3832
25	rs4918969	A	G	0.8426	0.02078	1E-200	4,128	469,372	0.0373	0.0224	0.0956489
26	rs5755717	C	A	0.1309	0.02956	9.80799E-06	4,128	469,372	-0.0171	0.0234	0.4666
27	rs61821998	A	T	0.4208	0.08415	6.01395E-07	4,128	469,372	0.181	0.0817	0.0266097
28	rs7310854	C	T	0.1246	0.02782	7.79704E-06	4,128	469,372	0.0288	0.0274	0.2928
29	rs77520132	A	G	0.6067	0.05819	4.4412E-25	4,128	469,372	-0.2007	0.1651	0.2241
30	rs78096816	C	A	1.581	0.3277	1.46599E-06	4,128	469,372	0.0801	0.0683	0.241
31	rs78654607	G	C	-0.3327	0.06909	1.52999E-06	4,128	469,372	-0.0039	0.0977	0.9683
32	rs78809247	T	C	-0.3668	0.08113	6.37397E-06	4,128	469,372	-0.0594	0.0776	0.4441
33	rs78843390	T	C	0.6146	0.1296	2.17701E-06	4,128	469,372	-0.0147	0.1017	0.8853
34	rs7963011	T	C	0.1209	0.02721	9.08699E-06	4,128	469,372	0.0054	0.0243	0.8236
35	rs884599	G	A	-0.1114	0.02473	0.000006844	4,128	469,372	-0.0054	0.0241	0.8235

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; SE, standard error; AA, aplastic anemia.

Supplementary Table S1.4 Information of identified SNPs in exposure (CD39+ secreting Treg AC) and outcomes (AA).

				Exposure (CD39+ secreting Treg AC)			Outcome (AA)				
	SNP	EA	OA	β	SE	*p* value	Case	Control	β	SE	*p* value
1	rs10786199	A	G	-0.1932	0.02906	3.39E-11	4,128	469,372	-0.0351	0.0228	0.1238
2	rs10882324	T	C	-0.1185	0.02675	9.67E-06	4,128	469,372	-0.0079	0.0249	0.7503
3	rs10882655	A	G	-0.9594	0.02046	1.00E-200	4,128	469,372	-0.0472	0.0224	0.0350897
4	rs10882701	C	A	-0.4069	0.02528	3.19E-56	4,128	469,372	-0.0002	0.0231	0.9941
5	rs111461378	C	T	-1.066	0.2404	9.47E-06	4,128	469,372	-0.0382	0.0936	0.6829
6	rs11188757	C	A	-0.2549	0.03174	1.33E-15	4,128	469,372	-0.0016	0.025	0.9495
7	rs11254133	T	C	-0.1723	0.03826	6.88E-06	4,128	469,372	-0.1155	0.0505	0.0221401
8	rs114114791	G	A	0.3538	0.07416	1.91E-06	4,128	469,372	-0.0009	0.0622	0.9885
9	rs11598645	C	T	-0.6337	0.1381	4.64E-06	4,128	469,372	-0.1392	0.068	0.0405098
10	rs11655883	G	A	0.1294	0.02794	3.74E-06	4,128	469,372	-0.0211	0.0285	0.457999
11	rs139577399	C	T	-0.2948	0.06509	6.14E-06	4,128	469,372	0.0287	0.1626	0.8598
12	rs149951966	C	T	0.2069	0.04388	2.51E-06	4,128	469,372	-0.0081	0.0289	0.7796
13	rs150315125	T	A	-0.4106	0.08147	4.89E-07	4,128	469,372	0.1189	0.1051	0.2582
14	rs202211780	G	A	0.4715	0.04614	3.68E-24	4,128	469,372	0.0553	0.077	0.4729
15	rs2836025	T	C	-0.2143	0.04774	7.40E-06	4,128	469,372	-0.0871	0.0413	0.0351399
16	rs56216441	T	A	-0.1636	0.03693	9.74E-06	4,128	469,372	-0.0261	0.0268	0.3291
17	rs57516532	T	C	0.3162	0.07091	8.48E-06	4,128	469,372	-0.022	0.0522	0.673701
18	rs6005121	G	A	-0.2011	0.04185	1.62E-06	4,128	469,372	-0.0152	0.0334	0.649201
19	rs7267949	T	C	-0.7497	0.1688	9.25E-06	4,128	469,372	-0.2565	0.1316	0.0512
20	rs72809762	T	C	0.4322	0.05126	4.98E-17	4,128	469,372	0.1145	0.0792	0.1482
21	rs74791100	C	T	0.5538	0.1185	3.09E-06	4,128	469,372	0.244	0.2032	0.2298
22	rs75270901	A	G	0.2585	0.05807	8.78E-06	4,128	469,372	-0.0548	0.0541	0.311
23	rs7570197	C	T	0.5826	0.1287	6.23E-06	4,128	469,372	0.0472	0.0567	0.4052
24	rs76464802	A	G	-0.4852	0.1056	4.47E-06	4,128	469,372	0.0043	0.0522	0.9343
25	rs76710109	C	T	-0.1594	0.03494	5.24E-06	4,128	469,372	0.0279	0.0344	0.4174
26	rs78027772	A	G	0.2257	0.04708	1.71E-06	4,128	469,372	-0.0538	0.0377	0.1538
27	rs7937686	G	C	0.1946	0.04346	7.81E-06	4,128	469,372	-0.0013	0.024	0.9572

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; SE, standard error; AA, aplastic anemia.

Supplementary Table S1.5 Information of identified SNPs in exposure (CD8 on CD28+ CD45RA- CD8br) and outcomes (AA).

				Exposure (CD8 on CD28+ CD45RA- CD8br)			Outcome (AA)				
	SNP	EA	OA	β	SE	*p* value	Case	Control	β	SE	*p* value
1	rs10181413	A	G	0.145	0.02796	2.28E-07	4,128	469,372	0.0295	0.0226	0.192
2	rs10185095	C	T	0.1228	0.02711	6.09E-06	4,128	469,372	-0.0289	0.0248	0.2431
3	rs117410826	T	G	0.2344	0.05245	8.18E-06	4,128	469,372	0.0547	0.1017	0.5907
4	rs12046688	G	A	0.187	0.03957	2.40E-06	4,128	469,372	0.0128	0.0272	0.6392
5	rs1235080	A	T	0.1343	0.02796	1.64E-06	4,128	469,372	-0.0165	0.0241	0.4923
6	rs13004867	G	C	-0.4974	0.04176	5.63E-32	4,128	469,372	-0.0633	0.0381	0.0965406
7	rs145728549	G	A	-0.8259	0.1843	7.69E-06	4,128	469,372	-0.0681	0.0778	0.3813
8	rs1616538	A	C	0.1532	0.03377	5.98E-06	4,128	469,372	-0.0597	0.0857	0.4856
9	rs34862764	T	C	-0.1258	0.02715	3.78E-06	4,128	469,372	-0.0003	0.0224	0.9883
10	rs4241667	G	A	-0.1342	0.02955	5.80E-06	4,128	469,372	0.005	0.0257	0.8469
11	rs56262516	A	G	0.2531	0.03508	6.75E-13	4,128	469,372	0.0611	0.0489	0.2117
12	rs58007649	A	G	0.1958	0.04087	1.76E-06	4,128	469,372	0.0072	0.0408	0.8598
13	rs59998168	C	T	0.131	0.02871	5.23E-06	4,128	469,372	0.0349	0.0286	0.2213
14	rs77892262	T	C	-0.2144	0.04156	2.65E-07	4,128	469,372	-0.1102	0.1153	0.3391
15	rs80304304	G	A	0.2387	0.05231	5.25E-06	4,128	469,372	0.0823	0.0385	0.0323303
16	rs9263601	C	T	0.2002	0.03074	8.61E-11	4,128	469,372	0.064	0.0255	0.01191

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; SE, standard error; AA, aplastic anemia.

Supplementary Table S1.6 Information of identified SNPs in exposure (CD86 on CD62L+ myeloid DC) and outcomes (AA).

				Exposure (CD86 on CD62L+ myeloid DC)			Outcome (AA)				
	SNP	EA	OA	β	SE	*p* value	Case	Control	β	SE	*p* value
1	rs112834744	T	C	0.7672	0.1459	1.57E-07	4128	469372	0.0053	0.0707	0.9402
2	rs112891649	A	G	0.2911	0.05373	6.54E-08	4128	469372	-0.0589	0.1166	0.613501
3	rs112932872	C	T	1.02	0.223	5.03E-06	4128	469372	-0.0983	0.047	0.0366201
4	rs116105787	T	A	1.124	0.2001	2.16E-08	4128	469372	-0.0964	0.0684	0.1589
5	rs116499957	T	C	-0.513	0.1113	4.21E-06	4128	469372	0.0921	0.0776	0.2354
6	rs12076726	T	C	0.2941	0.06566	7.76E-06	4128	469372	-0.0964	0.0468	0.0392004
7	rs139387290	A	C	-0.5368	0.1156	3.56E-06	4128	469372	-0.4423	0.1832	0.0157699
8	rs143092602	A	T	-0.785	0.1723	5.44E-06	4128	469372	0.0317	0.1645	0.8471
9	rs151105922	T	G	-0.3848	0.08691	9.86E-06	4128	469372	0.0502	0.0651	0.4406
10	rs2105590	A	G	-0.1461	0.03151	3.70E-06	4128	469372	0.0236	0.0245	0.3346
11	rs2737282	G	A	-0.1221	0.02754	9.57E-06	4128	469372	-0.0037	0.0256	0.8862
12	rs2835746	G	A	0.1372	0.03097	9.78E-06	4128	469372	0.0038	0.0236	0.8708
13	rs55989590	A	G	-0.2004	0.04064	8.66E-07	4128	469372	0.0145	0.0329	0.660601
14	rs56286676	C	G	-0.5253	0.1183	9.29E-06	4128	469372	0.4345	0.2058	0.0347296
15	rs62469564	T	C	0.8091	0.1679	1.53E-06	4128	469372	0.0224	0.0896	0.803
16	rs6830352	A	T	-0.2557	0.05756	9.28E-06	4128	469372	0.0196	0.051	0.6999
17	rs72771961	A	G	-0.2282	0.04734	1.51E-06	4128	469372	-0.2358	0.1057	0.0256803
18	rs7307939	C	T	-0.1213	0.0273	9.14E-06	4128	469372	0.0255	0.0241	0.2911

19	rs759125	G	A	0.2613	0.04814	6.16E-08	4128	469372	-0.0217	0.0425	0.609901
20	rs76607076	T	G	-0.1776	0.0392	6.08E-06	4128	469372	-0.0042	0.0286	0.8844
21	rs76831090	A	C	0.5003	0.1106	6.30E-06	4128	469372	-0.0659	0.1007	0.5128
22	rs9834116	C	A	-0.1233	0.0278	9.64E-06	4128	469372	-0.0098	0.0224	0.6605
23	rs9848900	A	G	-0.1823	0.03514	2.28E-07	4128	469372	0.0086	0.0253	0.7322

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; SE, standard error; AA, aplastic anemia.

Supplementary Table S1.7 Information of identified SNPs in exposure (CD86+ plasmacytoid DC AC) and outcomes (AA).

				Exposure (CD86+ plasmacytoid DC AC)			Outcome (AA)				
	SNP	EA	OA	β	SE	*p* value	Case	Control	β	SE	*p* value
1	rs10248630	G	T	-0.1133	0.02489	5.53E-06	4,128	469,372	-0.0281	0.0226	0.2126
2	rs112496823	C	A	0.1603	0.03305	1.29E-06	4,128	469,372	-0.0264	0.0363	0.4684
3	rs114468547	G	A	-0.2713	0.05921	4.77E-06	4,128	469,372	0.1705	0.1279	0.1826
4	rs11701450	G	C	0.3632	0.08004	5.90E-06	4,128	469,372	0.038	0.0636	0.550399
5	rs118148569	T	C	0.2729	0.05772	2.36E-06	4,128	469,372	0.0557	0.1259	0.6579
6	rs11852100	C	A	0.2639	0.0548	1.53E-06	4,128	469,372	-0.0316	0.0363	0.3844
7	rs13088702	C	T	0.3946	0.07957	7.45E-07	4,128	469,372	-0.0516	0.0386	0.1821
8	rs139557155	G	A	0.2575	0.05462	2.52E-06	4,128	469,372	-0.019	0.0893	0.8312
9	rs140090913	A	G	-1.091	0.2411	6.19E-06	4,128	469,372	0.0362	0.2492	0.8846
10	rs142245023	A	G	0.7025	0.1505	3.17E-06	4,128	469,372	-0.0498	0.0714	0.4851
11	rs150206669	G	C	-0.4937	0.1106	8.31E-06	4,128	469,372	-0.021	0.0649	0.7465
12	rs1991164	C	T	-0.117	0.02506	3.14E-06	4,128	469,372	0.0059	0.0226	0.7945
13	rs2725015	G	A	-0.1473	0.02834	2.17E-07	4,128	469,372	0.0353	0.0264	0.1816
14	rs313778	T	C	-0.2171	0.04867	8.45E-06	4,128	469,372	-0.0474	0.0633	0.4535
15	rs56164117	C	T	-0.1223	0.02608	2.87E-06	4,128	469,372	0.005	0.0237	0.8319
16	rs61852527	G	A	-0.2618	0.0509	2.84E-07	4,128	469,372	0.0792	0.099	0.4238
17	rs75070627	C	T	0.9624	0.1978	1.19E-06	4,128	469,372	-0.1217	0.0755	0.1068
18	rs78379738	G	T	0.3098	0.06801	5.42E-06	4,128	469,372	-0.0043	0.093	0.963
19	rs79116372	T	A	-0.3011	0.06551	4.44E-06	4,128	469,372	0.2073	0.0635	0.00109499
20	rs9513487	G	T	0.1234	0.0269	4.67E-06	4,128	469,372	0.0274	0.0232	0.238

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; SE, standard error; AA, aplastic anemia.

Supplementary Table S1.8 Information of identified SNPs in exposure (DC AC) and outcomes (AA).

				Exposure (DC AC)			Outcome (AA)				
	SNP	EA	OA	β	SE	*p* value	Case	Control	β	SE	*p* value
1	rs115102594	G	C	-0.252	0.05419	3.43E-06	4,128	469,372	0.053	0.032	0.0978295
2	rs115751481	A	T	-0.3782	0.08174	3.84E-06	4,128	469,372	-0.0014	0.0781	0.986
3	rs11748731	T	C	0.3124	0.06744	3.75E-06	4,128	469,372	-0.0825	0.0845	0.329
4	rs12144062	T	C	0.1591	0.03557	8.00E-06	4,128	469,372	0.0205	0.0324	0.527
5	rs138192280	T	C	0.1545	0.0336	4.40E-06	4,128	469,372	-0.0223	0.0274	0.4156
6	rs147431504	G	C	0.9392	0.2001	2.78E-06	4,128	469,372	-0.128	0.6245	0.8376
7	rs17017403	C	T	-0.195	0.04252	4.68E-06	4,128	469,372	0.0024	0.0353	0.946
8	rs17438107	T	A	0.1983	0.04369	5.88E-06	4,128	469,372	0.0334	0.0723	0.644401
9	rs191217950	A	G	0.6722	0.127	1.28E-07	4,128	469,372	-0.0884	0.1625	0.5864
10	rs2322660	T	C	0.1262	0.02779	5.77E-06	4,128	469,372	-0.0107	0.0251	0.6705
11	rs2490034	A	G	0.1328	0.02893	4.61E-06	4,128	469,372	0.0101	0.033	0.7595
12	rs28854187	A	G	0.1282	0.02716	2.45E-06	4,128	469,372	-2.00E-04	0.0243	0.994
13	rs35802809	T	G	-0.3287	0.07248	5.95E-06	4,128	469,372	0.0202	0.0811	0.8031
14	rs55971447	T	C	0.5889	0.02846	1.20E-89	4,128	469,372	-0.0622	0.0366	0.0897305
15	rs62504404	A	G	0.125	0.02491	5.48E-07	4,128	469,372	-0.026	0.0233	0.2648

16	rs6782857	A	G	0.1846	0.04063	5.74E-06	4,128	469,372	-0.0229	0.0497	0.6443
17	rs75072970	C	T	0.3483	0.05681	9.74E-10	4,128	469,372	0.031	0.065	0.6329
18	rs76168011	G	C	-0.7956	0.1796	9.74E-06	4,128	469,372	0.2204	0.1014	0.0296401
19	rs79275634	A	G	0.5945	0.1248	1.98E-06	4,128	469,372	-0.0766	0.0582	0.188
20	rs911228	A	G	0.1199	0.02567	3.09E-06	4,128	469,372	-0.0251	0.0246	0.3068
21	rs9590896	A	G	-0.1209	0.02728	9.58E-06	4,128	469,372	0.0079	0.0301	0.793

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; SE, standard error; AA, aplastic anemia.

Supplementary Table S1.9 Information of identified SNPs in exposure (DN (CD4-CD8-) NKT lymphocyte) and outcomes (AA).

				Exposure (DN (CD4-CD8-) NKT lymphocyte)			Outcome (AA)				
	SNP	EA	OA	β	SE	*p* value	Case	Control	β	SE	*p* value
1	rs11124150	G	A	-0.1185	0.02525	2.80E-06	4,128	469,372	0.0234	0.0232	0.3133
2	rs113413210	C	T	0.2508	0.0559	7.49E-06	4,128	469,372	-0.0361	0.0632	0.568499
3	rs115536607	T	C	0.3576	0.05416	4.64E-11	4,128	469,372	-0.0484	0.0665	0.4674
4	rs11579742	T	C	-0.5199	0.1161	7.79E-06	4,128	469,372	0.0988	0.064	0.123
5	rs11652532	A	G	-0.1515	0.03361	6.71E-06	4,128	469,372	-0.047	0.0298	0.1147
6	rs117147408	G	A	0.675	0.07557	6.50E-19	4,128	469,372	-0.1436	0.0604	0.0173201
7	rs11759822	T	G	0.1268	0.02693	2.58E-06	4,128	469,372	-0.0092	0.0249	0.7123
8	rs12792298	T	C	-0.3106	0.06573	2.38E-06	4,128	469,372	0.0667	0.1383	0.6297
9	rs140444563	G	T	0.1844	0.03996	4.09E-06	4,128	469,372	-0.0211	0.074	0.7753
10	rs148936961	T	A	0.367	0.0801	4.77E-06	4,128	469,372	0.028	0.0785	0.7217
11	rs149977848	A	G	0.4432	0.09784	6.09E-06	4,128	469,372	-0.0678	0.0757	0.3709
12	rs188455143	A	G	0.3487	0.07386	2.43E-06	4,128	469,372	-0.0223	0.1363	0.8699
13	rs192734627	A	G	0.306	0.06449	2.17E-06	4,128	469,372	-0.0509	0.0883	0.5639
14	rs201024668	T	A	0.2498	0.04992	5.90E-07	4,128	469,372	0.1976	0.1289	0.1252
15	rs265772	G	T	-0.2013	0.04454	6.39E-06	4,128	469,372	-0.0111	0.0456	0.8071
16	rs4233371	C	G	0.1528	0.03232	2.37E-06	4,128	469,372	-0.0176	0.036	0.6246
17	rs59209359	G	C	-0.224	0.04867	4.30E-06	4,128	469,372	0.0096	0.033	0.7712
18	rs61925057	T	C	-0.4258	0.09245	4.25E-06	4,128	469,372	0.046	0.0351	0.1908
19	rs6785704	G	A	-0.1997	0.04189	1.93E-06	4,128	469,372	0.0577	0.033	0.0800995
20	rs72784218	A	G	0.2338	0.0512	5.11E-06	4,128	469,372	0.0065	0.072	0.9286
21	rs73191859	G	A	0.1799	0.03903	4.16E-06	4,128	469,372	0.0348	0.0455	0.4441
22	rs7569868	T	C	0.2197	0.03135	2.88E-12	4,128	469,372	-0.0272	0.0284	0.3382
23	rs76158038	C	T	-0.2382	0.05267	6.28E-06	4,128	469,372	-0.0511	0.0481	0.2882
24	rs76333352	T	C	0.6311	0.1397	6.46E-06	4,128	469,372	0.1762	0.1269	0.1649
25	rs77738700	A	G	-0.3774	0.03905	7.85E-22	4,128	469,372	0.0685	0.0961	0.4758
26	rs79251210	A	G	0.5505	0.06465	2.39E-17	4,128	469,372	-0.0874	0.0713	0.2204
27	rs8054431	T	G	0.1122	0.0252	8.70E-06	4,128	469,372	0.0128	0.023	0.578699
28	rs9488834	A	G	0.3698	0.05821	2.39E-10	4,128	469,372	0.0682	0.1394	0.625
29	rs9509337	A	G	-0.1273	0.02859	8.73E-06	4,128	469,372	-0.0266	0.0251	0.2904
30	rs9916629	C	T	0.1378	0.02431	1.54E-08	4,128	469,372	0.0788	0.0652	0.2263
31	rs9983338	T	C	-0.416	0.09034	4.27E-06	4,128	469,372	0.0921	0.113	0.4152

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; SE, standard error; AA, aplastic anemia.

The IVW results showed that CD127- CD8br T cells [odds ratio (OR), 1.135; 95% confidence interval (CI), 1.032–1.247; p = 0.009], CD25 on IgD+ CD38dim (OR, 1.053; 95% CI, 1.013–1.095; p = 0.0091), CD39+ resting Treg CD4 Treg (OR, 1.034; 95% CI, 1.010–1.059; p = 0.005), CD39+ secreting Treg AC (OR, 1.050; 95% CI, 1.013–1.089; p = 0.007), and CD8 on CD28+ CD45RA- CD8br (OR, 1.127; 95% CI, 1.044–1.215; p = 0.002) were associated with increased genetic susceptibility to AA. Conversely, four types of immune cells were linked to decreased genetic susceptibility to AA, including CD86 on CD62L+ myeloid DC [odds ratio (OR), 0.930; 95% confidence interval (CI), 0.882–0.981; p = 0.008], CD86+ plasmacytoid DC AC (OR, 0.923;

95% CI, 0.857–0.994; p = 0.034), DC AC (OR, 0.897; 95% CI, 0.838–0.960; p = 0.002), and DN (CD4-CD8-) NKT lymphocyte (OR, 0.926; 95% CI, 0.869–0.986; p = 0.017). The forest plots are illustrated in **Fig. 1, Fig. 2, and Fig. 3**. MR-Egger indicated no significant horizontal pleiotropy in these results (p ≥ 0.05), as shown in **Supplementary Table S2**.

Supplementary Table S2 The results of MR-Egger intercept analysis.

Exposure	Outcome	Egger_intercept	SE	*p* value
CD127- CD8br %T cell	AA	-0.034810216	0.020347758	0.110865506
CD25 on IgD+ CD38dim	AA	-0.003742935	0.011218183	0.74180376
CD39+ resting Treg CD4 Treg	AA	0.001231412	0.008698836	0.888287789
CD39+ secreting Treg AC	AA	-0.004575161	0.012080676	0.708095059
CD8 on CD28+ CD45RA- CD8br	AA	-0.014100738	0.015909816	0.390430833
CD86 on CD62L+ myeloid DC	AA	0.005174919	0.012440516	0.68165001
CD86+ plasmacytoid DC AC	AA	0.01671713	0.014859181	0.275351926
DC AC	AA	0.008905599	0.014112327	0.535525448
DN (CD4-CD8-) NKT lymphocyte	AA	0.030667069	0.015116101	0.051751915

AA, aplastic anemia.

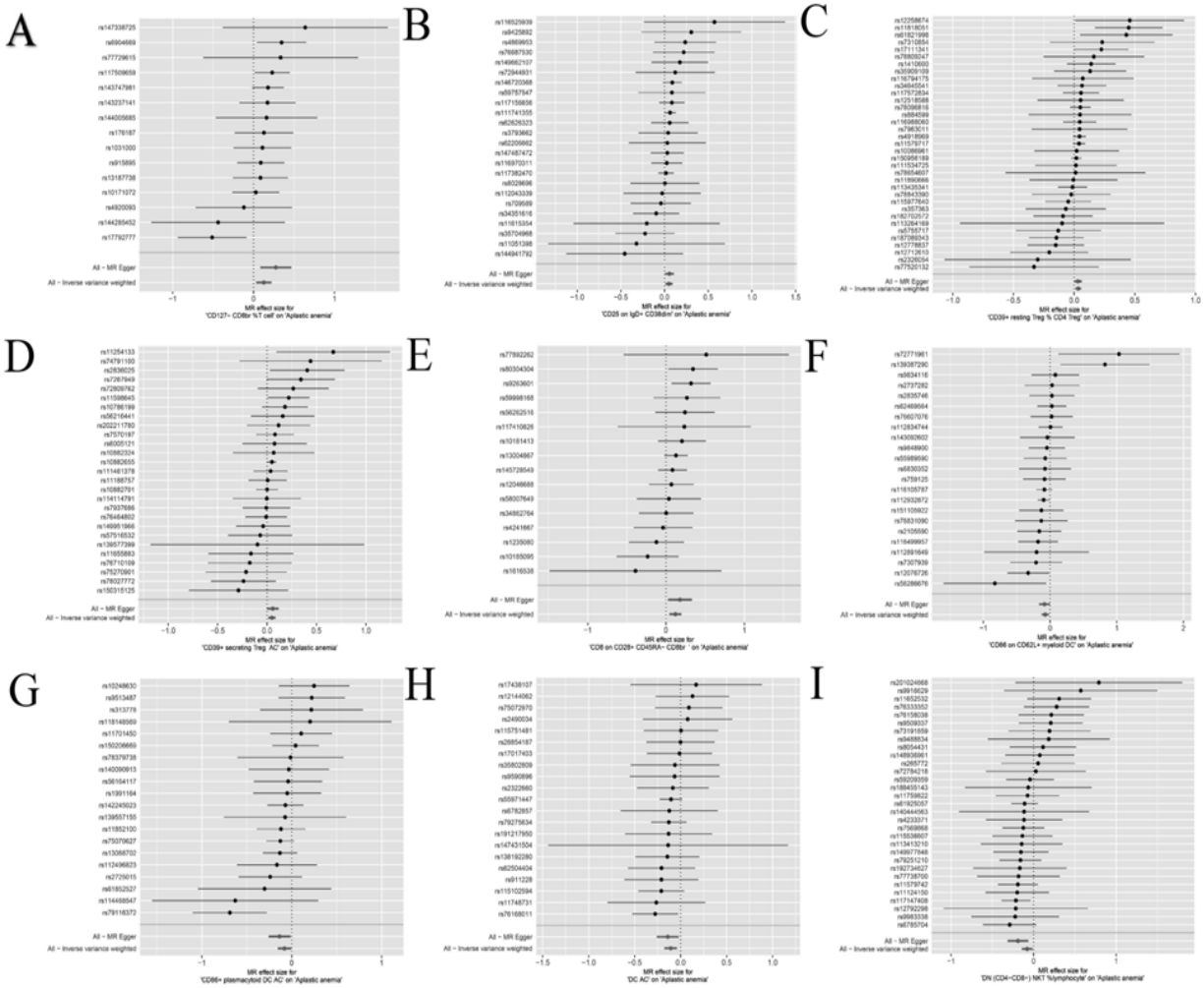

Figure 1. Assessing the relationship between immune cells and genetic susceptibility to aplastic anemia based on exposure factor-related instrumental variable evaluation. (A)CD127- CD8br T cell on AA. (B)CD25 on IgD+ CD38dim on AA. (C)CD39+ resting Treg CD4 Treg on AA. (D)CD39+ secreting Treg AC on AA. (E)CD8 on CD28+ CD45RA- CD8br on AA. (F)CD86 on CD62L+ myeloid DC on AA. (G)CD86+ plasmacytoid DC AC on AA. (H)DC AC on AA. (I)DN (CD4-CD8-) NKT lymphocyte on AA.

exposure	nsnp	method	pval	OR(95% CI)
CD127− CD8br %T cell	15	Weighted median	**0.007**	1.179 (1.045 to 1.330)
	15	Inverse variance weighted	**0.009**	1.135 (1.032 to 1.247)
CD25 on IgD+ CD38dim	24	Weighted median	**0.025**	1.064 (1.008 to 1.124)
	24	Inverse variance weighted	**0.009**	1.053 (1.013 to 1.095)
CD39+ resting Treg % CD4 Treg	35	Weighted median	**0.020**	1.039 (1.006 to 1.073)
	35	Inverse variance weighted	**0.005**	1.034 (1.010 to 1.059)
CD39+ secreting Treg AC	27	Weighted median	**0.037**	1.047 (1.003 to 1.093)
	27	Inverse variance weighted	**0.007**	1.050 (1.013 to 1.089)
CD8 on CD28+ CD45RA− CD8br	16	Weighted median	**0.047**	1.115 (1.001 to 1.241)
	16	Inverse variance weighted	**0.002**	1.127 (1.044 to 1.215)
CD86 on CD62L+ myeloid DC	23	Weighted median	**0.020**	0.917 (0.852 to 0.986)
	23	Inverse variance weighted	**0.008**	0.930 (0.882 to 0.981)
CD86+ plasmacytoid DC AC	20	Weighted median	**0.046**	0.904 (0.819 to 0.998)
	20	Inverse variance weighted	**0.034**	0.923 (0.857 to 0.994)
DC AC	21	Weighted median	**0.029**	0.893 (0.806 to 0.989)
	21	Inverse variance weighted	**0.002**	0.897 (0.838 to 0.960)
DN (CD4−CD8−) NKT %lymphocyte	31	Weighted median	**0.011**	0.886 (0.807 to 0.973)
	31	Inverse variance weighted	**0.017**	0.926 (0.869 to 0.986)

Figure 2. Summarried forest plot of immune cells that associated with aplastic anemia.

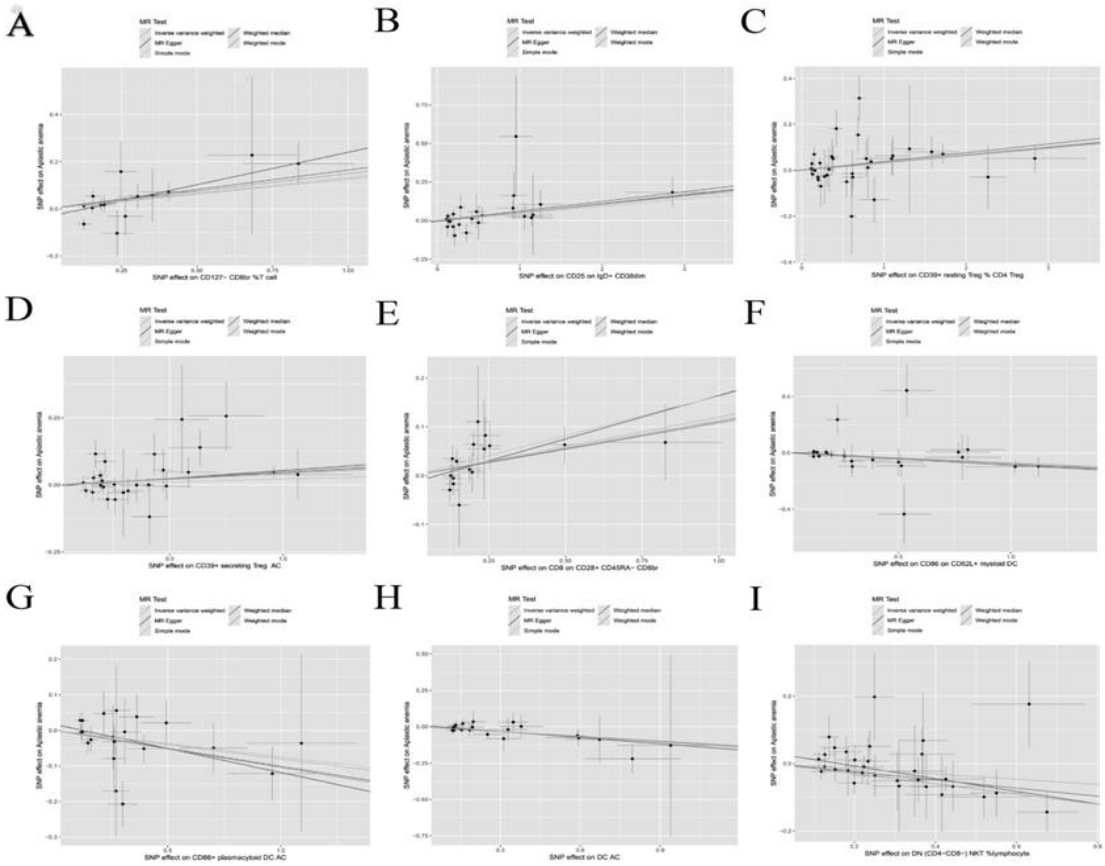

Figure 3. Scatter plot of MR analysis for immune cell phenotypes on genetic susceptibility to AA. (A)CD127- CD8br T cell on AA. (B)CD25 on IgD+ CD38dim on AA. (C)CD39+ resting Treg CD4 Treg on AA. (D)CD39+ secreting Treg AC on AA. (E)CD8 on CD28+ CD45RA- CD8br on AA. (F)CD86 on CD62L+ myeloid DC on AA. (G)CD86+ plasmacytoid DC AC on AA. (H)DC AC on AA. (I)DN (CD4-CD8-) NKT lymphocyte on AA. MR, Mendelian randomization; AA, aplastic anemia.

Heterogeneity and sensitivity analysis

Cochran's Q test revealed no heterogeneity in the MR analysis results ($p \geq 0.05$), as detailed in Supplementary Table S3 and Fig. 4. Sensitivity analysis demonstrated that the MR analysis results were robust, as illustrated in Fig. 5.

Supplementary Table S3 The results of Cochran's Q analysis.

Exposure	Method	Q	Q_df	Q_*p* val
CD127- CD8br %T cell	MR Egger	13.11734256	13	0.438789221
CD127- CD8br %T cell	Inverse variance weighted	16.07047432	14	0.309089458
CD25 on IgD+ CD38dim	MR Egger	13.6838798	22	0.912318098
CD25 on IgD+ CD38dim	Inverse variance weighted	13.79520139	23	0.932586121
CD39+ resting Treg % CD4 Treg	MR Egger	36.23112713	33	0.320260895
CD39+ resting Treg % CD4 Treg	Inverse variance weighted	36.25312865	34	0.363942141
CD39+ secreting Treg AC	MR Egger	28.42691102	25	0.288578355
CD39+ secreting Treg AC	Inverse variance weighted	28.58999825	26	0.330053017
CD8 on CD28+ CD45RA- CD8br	MR Egger	12.67147661	14	0.552539276
CD8 on CD28+ CD45RA- CD8br	Inverse variance weighted	13.45698961	15	0.567045017
CD86 on CD62L+ myeloid DC	MR Egger	23.83300057	21	0.301220329
CD86 on CD62L+ myeloid DC	Inverse variance weighted	24.02937716	22	0.345691394
CD86+ plasmacytoid DC AC	MR Egger	19.16805196	18	0.381544228
CD86+ plasmacytoid DC AC	Inverse variance weighted	20.51589462	19	0.364155446
DC AC	MR Egger	7.534147992	19	0.990775496
DC AC	Inverse variance weighted	7.932373413	20	0.992305778
DN (CD4-CD8-) NKT %lymphocyte	MR Egger	22.19728826	29	0.811929722
DN (CD4-CD8-) NKT %lymphocyte	Inverse variance weighted	26.31318973	30	0.659053992

AA, aplastic anemia.

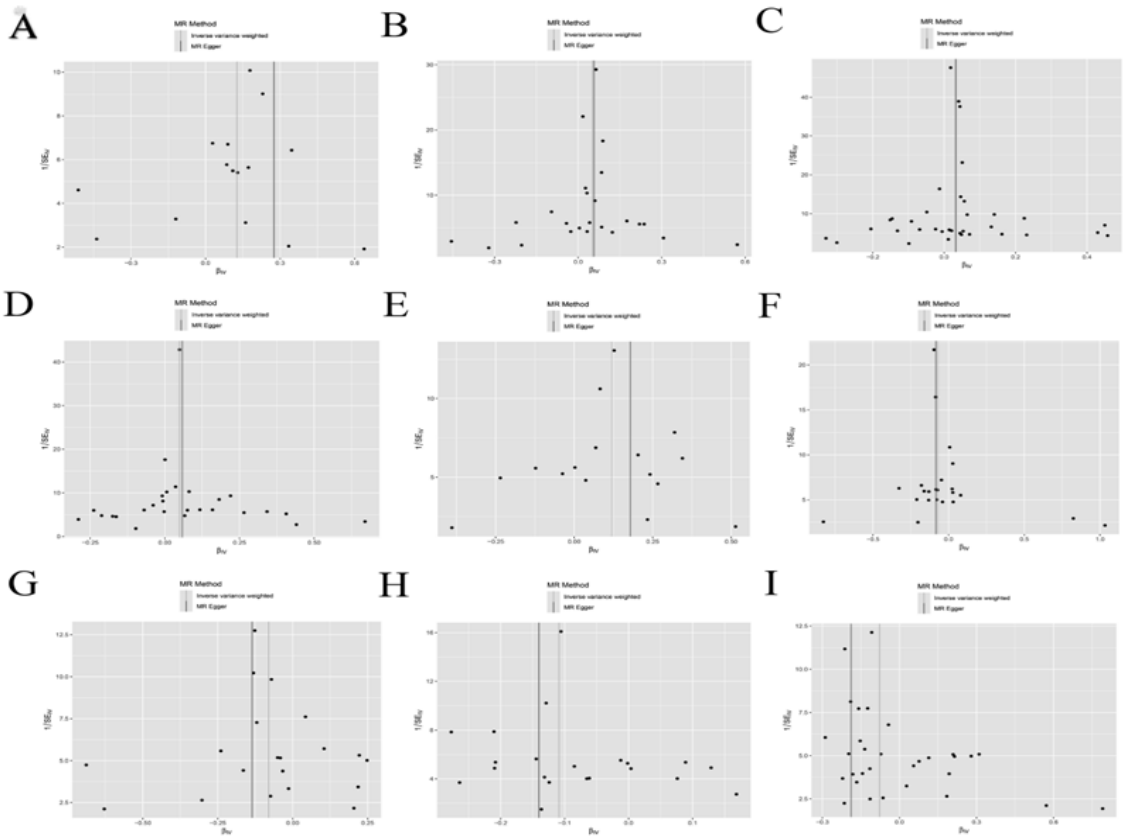

Figure 4. Funnel plot of MR analysis for immune cell phenotypes on genetic susceptibility to AA. (A)CD127- CD8br T cell on AA. (B)CD25 on IgD+ CD38dim on AA. (C)CD39+ resting Treg CD4 Treg on AA. (D)CD39+ secreting Treg AC on AA. (E)CD8 on CD28+ CD45RA- CD8br on AA. (F)CD86 on CD62L+ myeloid DC on AA. (G)CD86+ plasmacytoid DC AC on AA. (H)DC AC on AA. (I)DN (CD4-CD8-) NKT lymphocyte on AA. MR, Mendelian randomization; AA, aplastic anemia.

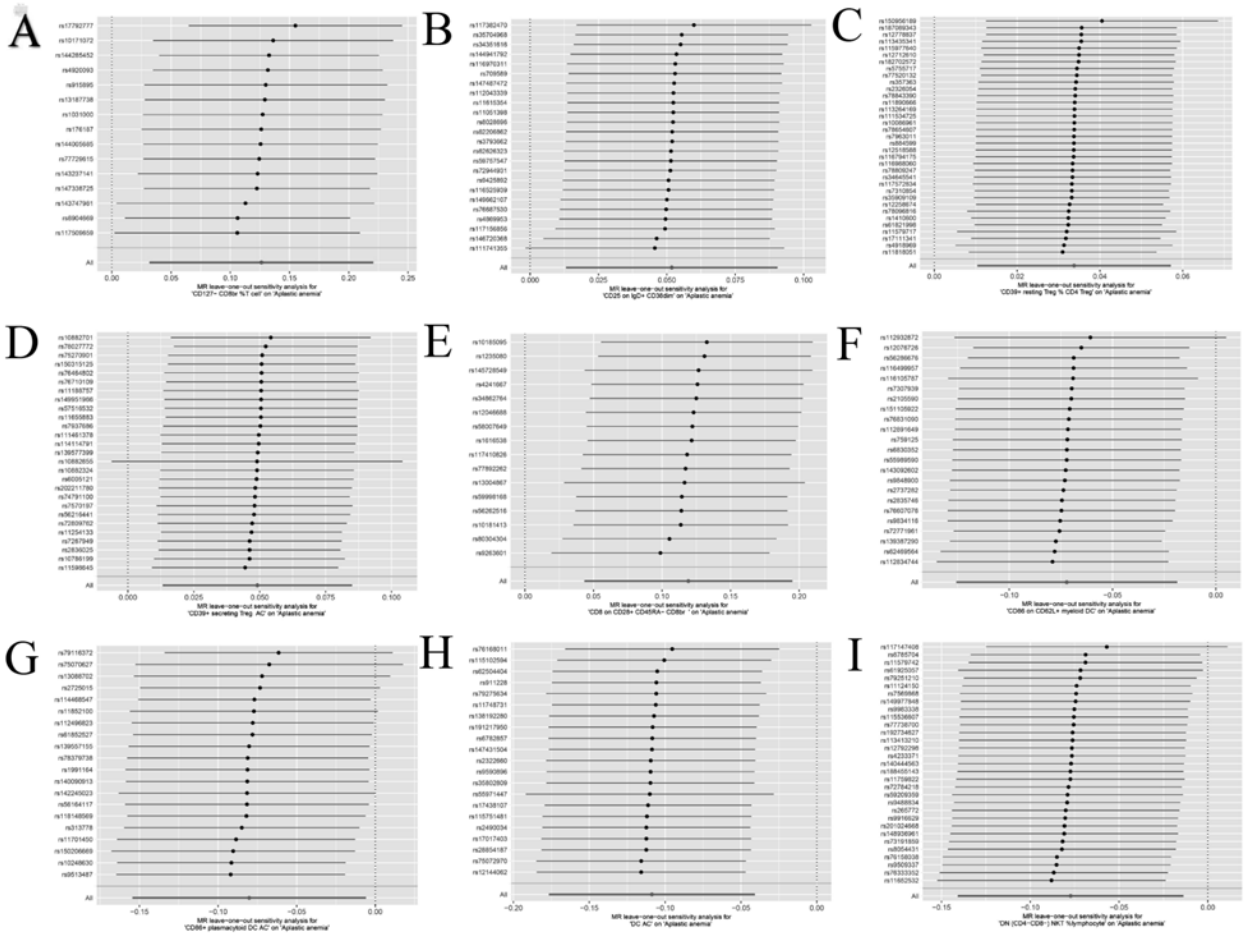

Figure 5. Leave-one-out sensitive analysis for immune cell phenotypes on genetic susceptibility to AA. (A)CD127- CD8br T cell on AA. (B)CD25 on IgD+ CD38dim on AA. (C)CD39+ resting Treg CD4 Treg on AA. (D)CD39+ secreting Treg AC on AA. (E)CD8 on CD28+ CD45RA- CD8br on AA. (F)CD86 on CD62L+ myeloid DC on AA. (G)CD86+ plasmacytoid DC AC on AA. (H)DC AC on AA. (I)DN (CD4-CD8-) NKT lymphocyte on AA. MR, Mendelian randomization; AA, aplastic anemia.

Discussion

Immune-related mechanisms are integral to the onset and progression of AA. In this study, we analyzed a substantial amount of publicly available genetic data to investigate the relationship between 731 types of immune cells and AA. According to existing literature, this is the first instance where Mendelian randomization has been applied to evaluate the genetic associations between numerous immune phenotypes and AA. Our findings revealed that nine immune phenotypes have a strong causal relationship with AA.

The results indicate that five types of immune cells act as risk factors for AA and may contribute to its development. These cells include CD127- CD8br T cells, CD8 on CD28+ CD45RA- CD8br, CD39+ resting Treg CD4 Treg, CD39+ secreting Treg AC,, and CD25 on IgD+ CD38dim. CD127- CD8br T cells refer to T cells with high CD8 expression and no CD127 expression. CD8 on CD28+ CD45RA- CD8br cells are T lymphocytes characterized by high CD8 expression, CD28 positivity, and CD45RA negativity. Currently, there are no reports linking these two specific immune phenotypes to genetic susceptibility to AA. These immune cell phenotypes primarily use CD8 as a marker, suggesting that CD8+ T cells play a pivotal role in the pathogenesis of AA. There is a significant body of research linking CD8+ T cells to AA. For example, as early as 2004, Zeng et al. reported that both innate and adaptive immune responses of CD8+ T cells might be active in immune-mediated bone marrow destruction [26]. Qi et al. found that EP300 and CREBBP levels increased in CD8+ T cells of patients with SAA and were associated with disease severity. They suggested that an imbalance of histone acetyltransferases and

histone deacetylases might activate CD8+ T cells, contributing to the immunopathogenesis of SAA [27-28]. Huang et al. indicated that CD8+ GITR+ T cells might negatively regulate excessive T cell activation in patients with aplastic anemia [29]. Additionally, You et al. discovered that CD8+ T cells from SAA patients include a highly activated CD38+ subset, which increases in the bone marrow of SAA patients and mouse models of SAA. This finding reveals a new pathway for regulating CD8+ T cell activation in SAA patients and suggests a potential therapeutic target for SAA treatment [30]. These studies collectively show that CD8+ T cells are closely linked to the pathogenesis of AA.

The current study demonstrated that two types of regulatory T cells are linked to an increased risk of AA, specifically CD39+ resting Treg CD4 Treg and CD39+ secreting Treg AC. CD39+ resting Treg CD4 Treg refers to regulatory T cells that are positive for CD39 and CD4 in a resting state. CD39+ secreting Treg AC represents the absolute count of CD39-positive secreting regulatory T cells. A recent Mendelian randomization study identified an association between CD39+ resting Treg CD4 Treg and the onset of gestational diabetes [31]. Lin et al. examined the role of regulatory T cells in children with AA and observed that, in comparison to the control group, the proportions of Tregs in peripheral blood and bone marrow lymphocytes were lower in the untreated AA group [32]. In patients with newly diagnosed very severe aplastic anemia (VSAA), the percentage of Tregs is lower than in healthy children, suggesting that Tregs could serve as a marker for therapeutic effects and prognosis in the treatment of childhood VSAA [33]. The study identified that increased levels of CD39+ secreting Treg AC were causally associated with the risk of systemic lupus erythematosus [34].

One type of B cell, identified as CD25 on IgD + CD38dim, was associated with a high risk of AA. According to the literature, there is no current literature linking CD25 on IgD+ CD38dim to the occurrence of AA. However, in newly diagnosed VSAA patients, the percentage of CD20+B cells is higher than in healthy children, indicating that CD20+B cells may be a therapeutic and prognostic marker for pediatric VSAA [35].

Four types of immune cells were associated with decreased genetic susceptibility to AA: CD86 on CD62L+ myeloid DC, CD86+ plasmacytoid DC AC, DC AC, and DN (CD4-CD8-) NKT lymphocyte. Among these, CD86 on CD62L+ myeloid DC, CD86+ plasmacytoid DC AC, and DC AC are subtypes of dendritic cells (DC). DCs can be classified into two forms: myeloid dendritic cells, which originate from myeloid stem cells upon stimulation by GM-CSF, and plasmacytoid dendritic cells, which derive from lymphoid stem cells. CD86 on CD62L+ myeloid DC refers to myeloid dendritic cells characterized by high CD86 expression and CD62L positivity. CD86+ plasmacytoid DC AC indicates the absolute count of CD86-positive plasmacytoid dendritic cells, while DC AC denotes the absolute count of dendritic cells. Studies have shown that patients with severe AA have higher levels of costimulatory molecules (CD80/b7-1, CD86/b7-2, CD40) on the surface of DCs compared to healthy individuals. The high expression of these molecules provides a second signal for T cell activation, initiating immune responses and reducing immune tolerance [14]. Most researchers believe that hyperactive DCs can abnormally activate T cells, potentially damaging the hematopoietic function of the bone marrow [36-38]. Sun et al. reported that increased phagocytic activity of myeloid dendritic cells in untreated severe aplastic anemia (SAA) patients may lead to abnormal activation of helper T cells and subsequent activation of cytotoxic T lymphocytes [39]. However, our study found that three types of dendritic cells are associated with decreased genetic susceptibility to AA, which contrasts with previous research findings. Literature suggests that the phenotype and function of DCs are highly affected by tissue localization and the inflammatory response. Factors such as location, activation status, and the surrounding environment can impact their functionality [40]. Other studies indicate that dendritic cells play a role in maintaining immune homeostasis, and targeting DCs to induce autoimmune tolerance has emerged as a new strategy for treating autoimmune diseases. Utilizing tolerant DCs to reduce T cell activation and maintain immune tolerance may be an effective approach to treating AA [41].

The DN (CD4-CD8-) NKT lymphocyte has been studied in various diseases. Chuang reported that

DN (CD4-CD8-) NKT lymphocytes can prevent airway hyperresponsiveness [42]. Wang et al., using Mendelian randomization analysis, found that (CD4-CD8-) NKT lymphocytes may be associated with schizophrenia [22]. Additionally, studies have shown that the frequency of CD4-CD8- (double-negative) natural killer T cells in the peripheral blood of β-thalassemia patients is significantly higher, indicating that these cells may play a role in the clinical progression of β-thalassemia major [43]. However, there have been limited studies on DN (CD4-CD8-) NKT in the context of AA to date.

The results obtained in this study are reliable, with horizontal pleiotropy and other potential confounding factors controlled through relevant parameters. However, our research also has limitations. Firstly, most of our data pertains to individuals of European ancestry, which may limit the applicability of our findings to Asian and other regional populations. Secondly, although multiple sensitivity analyses were performed to assess the assumptions of Mendelian randomization studies, it is impossible to completely eliminate the possibility of confounding bias and horizontal pleiotropy.

The role of immune cells in the pathogenesis of AA is highly significant for future research and clinical practice. Further investigation into the function of immune cells could guide the development of immunotherapy for AA. Future research might explore the dynamic changes in the composition of immune cells throughout the disease process and in response to different treatments. Stratifying patients based on their immune status may be crucial for developing effective treatment strategies.

This study examined the association between 731 immunophenotypes and genetic susceptibility to AA, identifying multiple immune cells linked to genetic susceptibility to AA. This provides a new perspective for studying the pathogenesis of AA.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the *IEU OpenGWAS project.*

Authors' Contributions

AGL,YTZ,XWP and JDL were responsible for collecting and analyzing data from public datasets, as well as performing statistical analysis. YHL contributed to the conception and design of the study, as well as providing language modification. All authors have reviewed and approved the final manuscript.

Ethics approval

Our study is based on open source data, so there are no ethical issues.

Patient consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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