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Causality Between 91 Circulating Inflammatory Proteins and Various Asthma Phenotypes: A Mendelian Randomization Study

Shiyao Zhang[1], Xiuying Zhang[2], Chenghao Wei[1], Lai Zhang[1], Zhaoyang Li[1]

[1]Liaoning University of Traditional Chinese Medicine, Shenyang, 110847, People's Republic of China; [2]The First Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Shenyang, 110032, People's Republic of China

Correspondence: Xiuying Zhang, Email pcxtcm@163.com

Objective: To investigate the causal relationship between 91 circulating inflammatory proteins and Various asthma phenotypes by means of Mendelian randomization.

Methods: Genome-wide association Studies (GWAS) of 91 inflammatory proteins were pooled from the Olink Target platform with 14,824 participants. Various asthma phenotypes were derived from the FinnGen Biobank. Inverse variance weighting (IVW) was used as the main method for MR Analysis, supplemented by Mr-Egger, Weighted median, Simple mode, and Weighted mode. The MR-Egger intercept term test and Cochran's Q test were used to test the polymorphism and heterogeneity of IVs, and visual analysis was carried out to draw scatter plots, funnel plots, and leave-out-one plots. The FDR correction was performed due to the possibility of a type 1 error.

Results: Genetically predicted IVW results revealed a total of 30 data sets suggesting a potential causal relationship between circulating inflammatory proteins and asthma phenotypes. Among them, 2 results were still strongly positive after FDR correction. The level of CST5 (OR=1.184; 95% CI: 1.075–1.305; P=0.0001; P-FDR=0.028) is associated with an increased risk of non-allergic asthma. LIF-R (OR=0.723; 95% CI: 0.620–0.842; P=0.000; P-FDR=0.003) is associated with a reduced risk of asthma in children. There was no pleiotropy or heterogeneity in the remaining 16 results that suggested a potential causal relationship.

Conclusion: Increased CST5 levels are associated with an increased risk of non-allergic asthma. LIF-R is associated with a reduced risk of asthma in children.

Keywords: mendelian randomization, inflammatory protein, asthma, childhood asthma, allergic asthma, non-allergic asthma, suggestive for eosinophilic asthma, obesity related asthma

Introduction

Asthma is one of the most common chronic respiratory diseases affecting children and adults worldwide. Despite significant reductions in mortality in some countries and regions, the global asthma mortality rate did not change significantly from 2006 to 2012, at about 0.19 deaths per 100,000 people (0.16–0.21).[1] There were approximately 81 million children with asthma worldwide in 2019, with an incidence of 1030.33 cases per 100,000 people (95% CI 683.66–1449.53).[2] Each phenotype and potential pathogenesis of asthma are different. Markers near the ORMDL3/GSDMB gene have been associated with childhood asthma, and interleukin (IL) 33 and IL1RL1SNP have been associated with atopic asthma. The thymic interstitial lymphoblastopoietin (TSLP) gene has been identified as a protective factor against TH2 asthma risk.[3]

In recent years, Personalized Medicine has made a multidisciplinary effort to better determine the different phenotypes of allergic diseases by endotyping their mechanisms.[4] Classifying diseases solely through phenotypes is insufficient to establish adequate or personalized treatments for patients since several phenotypes are superimposable and fluid, varying from one to the other.[5] Establishing the mechanisms responsible (endotypes) for the phenotypes is much more

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relevant, as it better demonstrates causality, being more appropriate for the personalized treatment of patients.[6] The laboratory tools currently available to the practicing clinician fall far short of the scientific knowledge already acquired, and efforts are being made to define endotype markers capable of aiding medical diagnosis. Historically, the classification of asthma phenotypes has evolved a lot. Asthma must be understood not as a single "disease" but as a phenotypic condition, a large "umbrella denomination" for several diseases of very different natures with completely different endotypes.[7] Even the simplistic classification into "allergic" and "non-allergic", "extrinsic", or "intrinsic" is fragile since, as knowledge advances in determining the mechanisms of hypersensitivity, more and more patients previously considered "not-allergic" are now being understood as "allergic", as tools for the diagnosis of non-IgE-mediated hypersensitivity are being developed.[8] In this sense, determining inflammatory markers related to the genotypic profile is crucial to assisting the endotype diagnosis, enriching the phenotypic classification, and helping to tailor the patient's treatment.

Mendelian randomization (MR) is a powerful tool for causal reasoning using single nucleotide polymorphisms (SNPs) as instrumental variables, relying on genome-wide association studies (GWAS) that utilize one or more genetic variants as instrumental variables (IVs) that are strongly associated with the exposure of interest and not influenced by confounding factors. The causal effects of exposure on results can be inferred. Previous studies have shown a significant relationship between inflammatory proteins and asthma phenotypes. Based on this, this study verified the relationship between 91 kinds of circulating inflammatory proteins and six asthma phenotypes through MR Analysis and public GWAS database summary statistics.

Materials and Methods

Study Design

MR Analysis must satisfy three key assumptions to obtain valid results. Specifically, to be used as instrumental variables (IVs) of an exposure factor, the genetic variation must be satisfied: (1) IVs establish a robust association with the studied exposure (correlation hypothesis); (2) IVs are independent of any confounding factors (independence assumption); (3) IVs affects outcomes only by exposure factors (excluding the limiting hypothesis).[9] Figure 1 depicts the study's overview in detail.

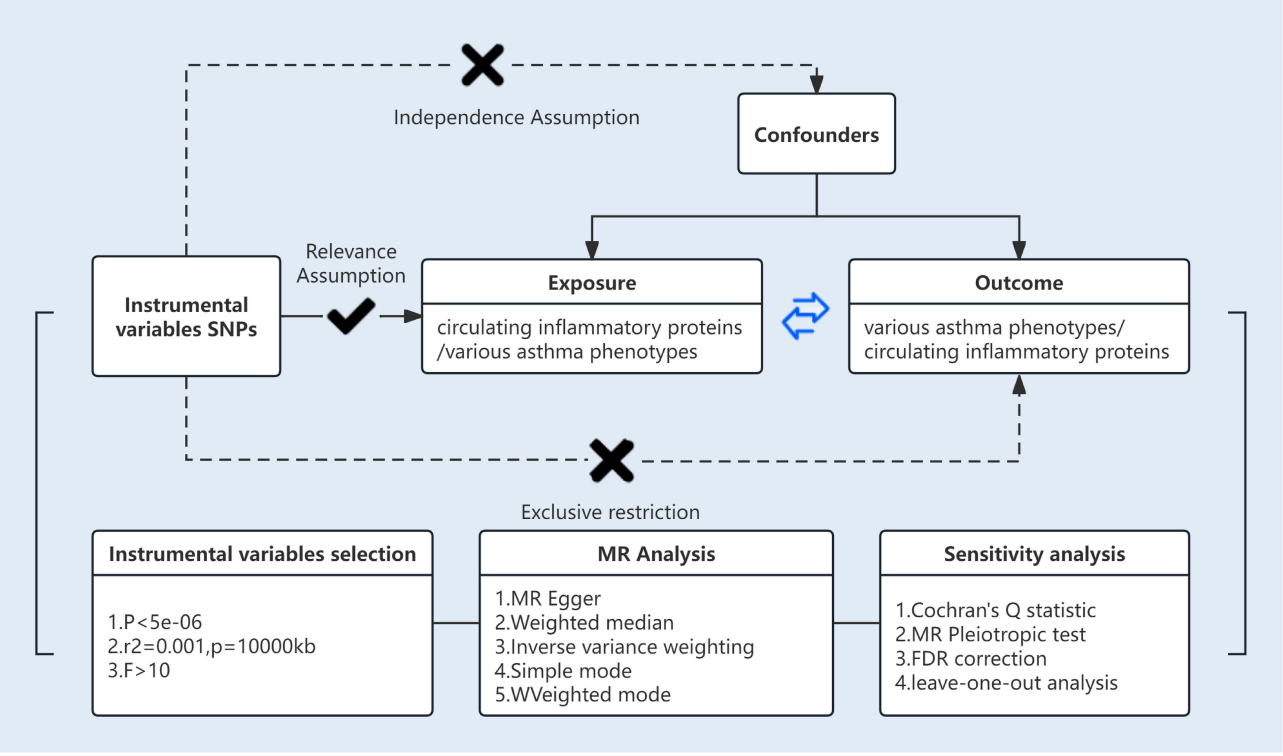

Figure 1 Overview of Mendelian randomization.

Data Sources

91 circulating inflammatory proteins were derived from a meta-analysis of 11 cohorts.[10] Complete protein GWAS summary statistics can be in https://www.phpc.cam.ac.uk/ceu/proteins and EBI GWAS directory (GCST90274758 - GCST90274848) to download. Various asthma phenotypes are derived from the FinnGen Consortium (R10)[11] database, as shown in Table 1. The population selection between the exposure group and the result group did not overlap, and the included populations were all European populations, meeting the requirement that both samples in MR Were from the same genetic background. Supplementary Table 1 summarizes the GWAS details of the 91 circulating inflammatory proteins included. And Supplementary Table 2 describes all SNPs information used as IVs. Our research methods and reports are based on the STROBE-MR guidelines, which are detailed in the Supplementary Table 3. The datasets used in this study were all drawn from publicly published GWAS databases and do not require ethical approval.

Instrumental Variables Selection

Firstly, SNPS of outcomes and 91 circulating inflammatory proteins were identified using a significance threshold of $p< 5\times10^{-8}$. However, for certain inflammatory proteins, the determination of the number of SNPS is limited under these conditions. In order to obtain more positive SNPS, we adjusted the threshold to 5×10^{-6} with reference to previously published papers.[12] Second, the SNPs were grouped together to eliminate linkage imbalances (kb = 10,000, $r^2= 0.001$).[13] During coordination, SNPS are excluded if they do not agree with the intermediate allele frequency or palindrome. Finally, we calculate the strength of each SNP through the F statistic. Using the following formulas:

$$F = \frac{R^2 \times (N - 1 - K)}{K \times (1 - R^2)}$$

$$R^2 = \frac{2 * EAF * (1 - EAF) * \beta^2}{2 * EAF * (1 - EAF) * \beta^2 + 2 * EAF * (1 - EAF) * N * S_{\bar{x}}^2}$$

K is the number of instrumental variables included. R^2 represents the proportion of variance in phenotype explained by a single SNP. N represents the sample size, Beta represents the estimated genetic effect of the SNPs on exposure, EAF represents the effect of allele frequency, and represents the standard error of Beta.[14] SNPS with F statistic >10 are considered to have strong correlation.[15] So if F statistic <10, they are excluded. The outlier SNPS that affect the level pluripotency are eliminated by the outlier test of the MR-PRESSO method.[16]

MR Analysis

IVW was used as the main research method. MR-Egger, Weighted median method, Simple mode method, and Weighted mode method are used as supplements. Results were presented as OR and 95% CI and $p<0.05$ was considered statistically consistent. First, in the absence of either heterogeneity or pleiotropy, the IVW estimate was chosen as the preferred option. Secondly, in the case of heterogeneity but no pleiotropy, the weighted median method is the preferred analysis method.

Table 1 Detailed Information for the Various Asthma Phenotypes Data

Trait	GWAS ID	Year	Population	Sample Size	Number of SNPs
Asthma	finn-b-J10_ASTHMA	2021	European	37253	16,380,176
Allergic asthma	finn-b-ALLERG_ASTHMA	2021	European	8525	16,379,987
Non-allergic asthma	finn-b-ASTHMA_NONALLERG	2021	European	6390	16,380,374
Childhood asthma	finn-b-ASTHMA_CHILD	2021	European	5282	16,379,865
Suggestive for eosinophilic asthma	finn-b-ASTHMA_EOSINOPHIL_SUGG	2021	European	2179	16,379,830
Obesity related asthma	finn-b-ASTHMA_OBESITY	2021	European	8774	16,379,879

Sensitivity Analysis

Heterogeneity among IVs was evaluated by Cochran's Q statistic. MR Pleiotropic test was used for MR-Egger and intercept value was returned to evaluate horizontal pleiotropic. Since 91 inflammatory proteins were examined, we applied FDR correction to reduce the incidence of Class I errors. A positive result means that the difference is significant before correction ($p<0.05$), and the difference is still significant after correction ($p<0.05$). It was significant before correction ($p<0.05$), but not significant after FDR correction, indicating a suggestive association result with potential causality.[17] A sensitivity analysis called "leave-one-out" was conducted by leaving out one SNP at a time to assess if a single SNP had a disproportionate effect on the overall estimation. All analyses were performed using the TwoSampleMR package in R4.3.3

Result

Instrumental Variables result

After the clumped function was used to remove the linkage disequilibrium, a total of 1817 SNPs remained for 91 inflammatory proteins. After calculation, it is concluded that the F-value corresponding to each single SNP in this study is greater than 10, that is, there is no weak instrumental variable bias.

MR Analysis

Forward MR Analysis results

The specific results of forward MR Analysis are shown in Table 2. The results showed that CD40, IL-8, CD6, and TNFRSF9 had a potential causal relationship with asthma. Higher genetically determined CD40 levels (increased 1-SD) were found to be associated with a reduced chance of asthma by the IVW approach, with a 6.6% reduction in asthma risk for each additional 1 SD (OR=0.934; 95% CI: 0.886–0.984; P = 0.010); According to the weighted median method (OR=0.932; 95% CI: 0.877–0.991; P=0.043). Elevated levels of IL-8, CD6, and TNFRSF9 were associated with increased asthma risk, with each additional SD increasing by 12.2% (OR=1.122; 95% CI: 1.023–1.230; P=0.014), 7.9% (OR=1.079; 95% CI: 1.017–1.145; P=0.011) and 7.1% (OR=1.071; 95% CI: 1.000–1.146; P=0.050). Scatter plots, funnel plots, and leave plots of MR Analyses of potentially causal inflammatory factors and asthma are shown in Supplemental Figure S1. There was no statistical significance between the intercept term of the above inflammatory factor MR-Egger and 0 (P>0.05), indicating that there was no horizontal pleiotropy. Cochran's Q test did not observe the statistical significance of heterogeneity among SNPS (P>0.05).

Reverse MR Analysis Results

The reverse MR Analysis results are shown in Table 3. The results showed that asthma was positively correlated with CD40 and CD6 levels ($P < 0.05$). There was no significant causal relationship between asthma and IL-8 and TNFRSF9 ($P > 0.05$).

Table 2 MR Analysis Results of Causal Association Between Inflammatory Proteins and Asthma

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-FDR*	*P-Qtest*	*P-intercept*
CD40	Asthma	IVW	16	0.934	0.886	0.984	0.010	0.915	0.405	
		MR Egger	16	0.927	0.858	1.002	0.076		0.339	0.813
IL-8	Asthma	IVW	17	1.122	1.023	1.230	0.014	0.435	0.532	
		MR Egger	17	1.219	1.014	1.466	0.052		0.537	0.323
CD6	Asthma	IVW	23	1.079	1.017	1.145	0.011	0.518	0.065	
		MR Egger	23	1.060	0.972	1.156	0.208		0.053	0.579
TNFRSF9	Asthma	IVW	27	1.071	1.000	1.146	0.050	0.903	0.176	
		MR Egger	27	1.013	0.870	1.181	0.865		0.165	0.438

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

Table 3 Reverse MR Analysis Results of Causal Association Between Inflammatory Proteins and Asthma

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	P	P-Qtest	P-Intercept
Asthma	CD40	IVW	62	1.055	1.010	1.102	0.016	0.534	
		MR Egger	62	1.086	0.969	1.218	0.163	0.508	0.593
Asthma	IL-8	IVW	62	1.022	0.977	1.068	0.350	0.652	
		MR Egger	62	0.951	0.846	1.069	0.403	0.677	0.200
Asthma	CD6	IVW	26	1.069	1.017	1.124	0.008	0.097	
		MR Egger	62	1.068	0.937	1.218	0.327	0.083	0.990
Asthma	TNFRSF9	IVW	62	1.031	0.971	1.094	0.322	0.012	
		MR Egger	62	0.994	0.851	1.162	0.942	0.011	0.625

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

Subgroup MR Analysis

91 Circulating Inflammatory Proteins and Allergic Asthma

As shown in Table 4, a total of 4 possible causal relationships were identified between the circulating inflammatory proteins and the risk of developing allergic asthma. The reverse MR Analysis results are shown in Table 5.

In the MR analysis, the level of IL-2 (OR=1.193; 95% CI: 1.018–1.398; P=0.029, P-FDR=2.640) was significantly associated with an increased risk of allergic asthma. And levels of SCF (OR=0.910; 95% CI: 0.833–0.995; P=0.039, P-FDR=1.183), TRAIL (OR=0.909; 95% CI: 0.831–0.994; P=0.036, P-FDR=1.640), TWEAK (OR=0.838; 95% CI: 0.704–0.997; P=0.046, P-FDR=1.050) was associated with a reduced risk of allergic asthma. According to Cochran's Q-test, there was no evidence of heterogeneity in the IVW model (Table 4). As anticipated, no evidence of horizontal pleiotropy was found according to the MR-Egger intercept (Table 4). Heterogeneity and sensitivity analysis results

Table 4 Effects of the Relationship Between Meaningful Circulating Inflammatory Proteins and Allergic Asthma in MR Analysis

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-FDR*	*P-Qtest*	*P-intercept*
IL-2	Allergic asthma	IVW	16	1.193	1.018	1.398	0.029	2.640	0.528	
		MR Egger	16	1.082	0.719	1.629	0.712		0.472	0.619
SCF	Allergic asthma	IVW	33	0.910	0.833	0.995	0.039	1.183	0.403	
		MR Egger	33	0.960	0.819	1.126	0.621		0.386	0.433
TRAIL	Allergic asthma	IVW	26	0.909	0.831	0.994	0.036	1.640	0.211	
		MR Egger	26	0.942	0.815	1.089	0.426		0.189	0.541
TWEAK	Allergic asthma	IVW	17	0.838	0.704	0.997	0.046	1.050	0.646	
		MR Egger	17	0.952	0.601	1.508	0.837		0.601	0.565

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

Table 5 Effects of the Relationship Between Meaningful Allergic Asthma and Circulating Inflammatory Proteins in Reverse MR Analysis

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-Qtest*	*P-intercept*
Allergic asthma	IL-2	IVW	29	1.028	0.973	1.085	0.331	0.023	
		MR Egger	29	0.989	0.847	1.155	0.888	0.019	0.608
Allergic asthma	SCF	IVW	29	0.977	0.940	1.016	0.248	0.663	
		MR Egger	29	0.939	0.841	1.049	0.278	0.642	0.460
Allergic asthma	TWEAK	IVW	29	0.990	0.941	1.042	0.712	0.087	
		MR Egger	29	0.983	0.850	1.136	0.813	0.069	0.908
Allergic asthma	TRAIL	IVW	29	0.989	0.950	1.029	0.571	0.399	
		MR Egger	29	1.079	0.966	1.205	0.191	0.488	0.111

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

corroborated the accuracy of the results. Scatter plots and funnel plots also supported these findings (Supplementary Figure S2). The leave-one-out method further validated the robustness of the data (Supplementary Figure S2). It is also regrettable that there were no significant positive results after the FDR correction.

91 Circulating Inflammatory Proteins and Non-Allergic Asthma

IVW analysis revealed 6 possible causal relationships between the circulating inflammatory proteins and the risk of non-allergic asthma (Table 6). The reverse MR Analysis results are shown in Table 7.The level of CCL19 (OR=1.140; 95% CI: 1.011–1.286; P=0.033; P-FDR=0.592), CST5 (OR=1.184; 95% CI: 1.075–1.305; P=0.0001; P-FDR=0.028), IL-7 (OR=1.272; 95% CI: 1.017–1.591; P=0.035; P-FDR=0.534) was significantly associated with an increased risk of allergic asthma. The level of CX3CL1 (OR=0.731; 95% CI: 0.611–0.874; P=0.0001; P-FDR=0.055), LAP TGF-beta-1 (OR=0.797; 95% CI: 0.669–0.950; P=0.011; P-FDR=0.342), ST1A1 (OR=0.870; 95% CI: 0.766–0.988; P=0.032; P-FDR=0.731) were associated with reduced risk of non-allergic asthma. Among them, there were significant positive results of CST5 after FDR correction, suggesting that CST5 levels were associated with an increase in non-allergic asthma. No significant heterogeneity or pleiotropy was found in Cochran's Q-test, MR-Egger intercept, heterogeneity, and sensitivity analysis (Table 6). Scatter plots, funnel plots, and leave-one-out plots are shown results in Supplementary

Table 6 Effects of the Relationship Between Meaningful Circulating Inflammatory Proteins and Non-Allergic Asthma in MR Analysis

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-FDR*	*P-Qtest*	*P-intercept*
CCL19	Non-allergic asthma	IVW	19	1.140	1.011	1.286	0.033	0.592	0.972	
		MR Egger	19	1.127	0.943	1.348	0.206		0.958	0.869
CST5	Non-allergic asthma	IVW	32	1.184	1.075	1.305	0.001	0.028	0.667	
		MR Egger	32	1.284	1.106	1.490	0.003		0.716	0.175
CX3CL1	Non-allergic asthma	IVW	22	0.731	0.611	0.874	0.001	0.055	0.558	
		MR Egger	22	0.661	0.429	1.018	0.075		0.511	0.621
IL-7	Non-allergic asthma	IVW	15	1.272	1.017	1.591	0.035	0.534	0.369	
		MR Egger	15	0.882	0.498	1.565	0.676		0.427	0.199
LAP-TGF-beta-1	Non-allergic asthma	IVW	19	0.797	0.669	0.950	0.011	0.342	0.325	
		MR Egger	19	0.721	0.532	0.977	0.050		0.305	0.435
ST1A1	Non-allergic asthma	IVW	23	0.870	0.766	0.988	0.032	0.731	0.787	
		MR Egger	23	1.077	0.791	1.465	0.643		0.854	0.151

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

Table 7 Effects of the Relationship Between Meaningful Non-Allergic Asthma and Circulating Inflammatory Proteins in Reverse MR Analysis

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-Qtest*	*P-intercept*
Non-allergic asthma	CCL19	IVW	10	1.002	0.940	1.067	0.963	0.090	
		MR Egger	10	0.997	0.874	1.137	0.963	0.059	0.936
Non-allergic asthma	CST5	IVW	10	1.033	0.973	1.097	0.289	0.122	
		MR Egger	10	1.055	0.933	1.193	0.418	0.089	0.705
Non-allergic asthma	CX3CL1	IVW	10	0.981	0.935	1.030	0.441	0.650	
		MR Egger	10	0.970	0.883	1.065	0.540	0.559	0.782
Non-allergic asthma	IL-7	IVW	10	0.992	0.944	1.041	0.734	0.694	
		MR Egger	10	1.015	0.922	1.117	0.771	0.631	0.595
Non-allergic asthma	LAP TGF-beta-1	IVW	10	0.989	0.926	1.056	0.749	0.059	
		MR Egger	10	0.994	0.868	1.138	0.930	0.037	0.942
Non-allergic asthma	ST1A1	IVW	10	0.979	0.924	1.037	0.472	0.361	
		MR Egger	10	0.984	0.874	1.107	0.790	0.275	0.932

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

Figure S3. Subsequently, different colors in Table 6 represent the results for each meaningful circulating inflammatory protein.

91 Circulating Inflammatory Proteins and Suggestive for Childhood Asthma

ARTN, CCL25, IL-18R1, LIF-R, and TNF were all potentially causally associated with childhood asthma (<16 years), in which LIF-R was significantly positive (P-FDR=0.003). Higher genetically determined ARTN and IL-18R1 levels were associated with an increased risk of asthma in children (<16 years of age) by IVW, with each additional SD increasing the risk of asthma by 25.2% (OR=1.252; 95% CI: 1.017–1.542; P=0.034) and 11.1% (OR=1.111; 95% CI: 1.007–1.227; P=0.036). CCL25, LIF-R, and TNF levels were associated with reduced asthma risk in children, with each increase of 1 SD associated with a 1.4% decrease in asthma risk (OR=0.869; 95% CI: 0.776 to 0.972; P=0.014), 2.7% (OR=0.723; 95% CI: 0.620–0.842; P=0.000) and 1.5% (OR=0.845; 95% CI: 0.725–0.985; P=0.031). MR Analysis scatterplot, funnel plot, and left plot are shown in supplementary Figure S4. No evidence of horizontal pleiotropy was found at the MR-Egger intercept (Table 8). Heterogeneity and sensitivity analysis confirmed the accuracy of the results. The reverse MR Analysis results are shown in Table 9.

91 Circulating Inflammatory Proteins and Suggestive for Eosinophilic Asthma

Four possible causal relationships were found between the circulating inflammatory proteins and suggestive of eosinophilic asthma. The level of CXCL6 (OR=1.246; 95% CI: 1.019–1.524; P=0.032; P-FDR=0.970), CST5 (OR=1.209; 95% CI: 1.005–1.453; P=0.044; P-FDR=1.002), SLAMF1 (OR=1.318; 95% CI: 1.059–1.641; P=0.013; P-FDR=1.223) suggest a potential causal relationship with increased the risk of suggestive for eosinophilic asthma. The level of

Table 8 Results of MR Analysis Between Causally Associated Inflammatory Proteins and Asthma in Children (<16 y)

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-FDR*	*P-Qtest*	*P-intercept*
ARTN	Asthma in children	IVW	20	1.252	1.017	1.542	0.034	0.770	0.249	
		MR Egger	20	1.661	0.976	2.827	0.078		0.268	0.274
CCL25	Asthma in children	IVW	24	0.869	0.776	0.972	0.014	0.660	0.090	
		MR Egger	24	0.872	0.732	1.040	0.141		0.069	0.955
IL-18R1	Asthma in children	IVW	22	1.111	1.007	1.227	0.036	0.658	0.227	
		MR Egger	22	1.050	0.902	1.223	0.533		0.228	0.348
LIF-R	Asthma in children	IVW	16	0.723	0.620	0.842	0.000	0.003	0.497	
		MR Egger	16	0.611	0.457	0.818	0.005		0.557	0.206
TNF	Asthma in children	IVW	27	0.845	0.725	0.985	0.031	0.951	0.417	
		MR Egger	27	0.718	0.513	1.005	0.065		0.424	0.298

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

Table 9 Effects of the Relationship Between Meaningful Asthma in Children and Circulating Inflammatory Proteins in Reverse MR Analysis

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-Qtest*	*P-intercept*
Asthma in children	ARTN	IVW	26	0.986	0.956	1.017	0.376	0.428	
	ARTN	MR Egger	26	1.022	0.955	1.094	0.532	0.448	0.254
Asthma in children	CCL25	IVW	26	0.989	0.961	1.019	0.476	0.304	
	CCL25	MR Egger	26	1.010	0.947	1.077	0.759	0.281	0.482
Asthma in children	IL-18R1	IVW	25	0.983	0.950	1.018	0.344	0.052	
	IL-18R1	MR Egger	25	1.006	0.932	1.084	0.887	0.046	0.518
Asthma in children	LIF-R	IVW	26	0.982	0.952	1.014	0.591	0.215	
	LIF-R	MR Egger	26	0.978	0.914	1.046	0.603	0.208	0.398
Asthma in children	TNF	IVW	26	0.991	0.959	1.024	0.639	0.927	
	TNF	MR Egger	26	0.987	0.923	1.056	0.996	0.945	0.230

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

Table 10 Effects of the Relationship Between Meaningful Circulating Inflammatory Proteins and Suggestive for Eosinophilic Asthma in MR Analysis

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-FDR*	*P-Qtest*	*P-intercept*
CXCL6	Suggestive for eosinophilic asthma	IVW	15	1.246	1.019	1.524	0.032	0.970	0.323	
		MR Egger	15	1.135	0.820	1.570	0.463		0.290	0.481
CST5	Suggestive for eosinophilic asthma	IVW	32	1.209	1.005	1.453	0.044	1.002	0.093	
		MR Egger	32	1.157	0.867	1.544	0.329		0.078	0.702
SLAMF1	Suggestive for eosinophilic asthma	IVW	28	1.318	1.059	1.641	0.013	1.223	0.603	
		MR Egger	28	1.011	0.624	1.639	0.965		0.631	0.238
TNFB	Suggestive for eosinophilic asthma	IVW	25	0.834	0.709	0.982	0.030	1.350	0.156	
		MR Egger	25	0.731	0.570	0.938	0.022		0.194	0.186

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

Table 11 Effects of the Relationship Between Meaningful Suggestive for Eosinophilic Asthma and Circulating Inflammatory Proteins in Reverse MR Analysis

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-Qtest*	*P-intercept*
suggestive for eosinophilic asthma	CST5	IVW	19	0.995	0.976	1.015	0.645	0.430	
		MR Egger	19	0.973	0.938	1.009	0.162	0.500	0.170
suggestive for eosinophilic asthma	CXCL6	IVW	19	1.014	0.994	1.035	0.160	0.915	
		MR Egger	19	1.019	0.981	1.058	0.352	0.885	0.805
suggestive for eosinophilic asthma	SLAMF1	IVW	19	1.018	0.997	1.038	0.087	0.556	
		MR Egger	19	1.007	0.970	1.046	0.724	0.517	0.526
suggestive for eosinophilic asthma	TNFB	IVW	18	1.025	0.998	1.052	0.070	0.172	
		MR Egger	18	1.002	0.954	1.052	0.946	0.185	0.298

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

TNFB (OR=0.834; 95% CI: 0.709–0.982; P=0.030; P-FDR=1.350) was associated with a reduced risk of suggestive eosinophilic asthma. Unfortunately, there were no significant positive circulating inflammatory proteins after FDR correction. The results of MR-Egger and MR-PRESSO tests confirmed that there is no horizontal pleiotropy and the outcomes from Cochrane's Q test demonstrated that there is no obvious heterogeneity among the selected SNPs (Table 10). Scatter plots, funnel plots, and leave-one-out plots are shown results in Supplementary Figure S5. The reverse MR Analysis results are shown in Table 11.

91 Circulating Inflammatory Proteins and Suggestive for Obesity Related Asthma

IVW analysis revealed 3 possible causal relationships between the 91 circulating inflammatory proteins and the risk of obesity-related asthma. After the FDR correction, there was no significant strong correlation. There is only a potential causal relationship between the three inflammatory proteins associated with obesity-related asthma. The level of FGF-5 (OR=1.104; 95% CI: 1.018–1.198; P=0.017; P-FDR=0.790), IL-17A (OR=1.235; 95% CI: 1.021–1.493; P=0.030; P-FDR=0.900) suggests a potential causal relationship with increased the risk of obesity-related asthma. The level of PD-L1 (OR=0.781; 95% CI: 0.663–0.922; P=0.003; P-FDR=0.310) was associated with a reduced risk of obesity related asthma. No significant heterogeneity or pleiotropy was found in Cochran's Q-test, MR-Egger intercept, heterogeneity, and sensitivity analysis (Table 12). Scatter plots, funnel plots, and leave-one-out plots are shown results in Supplementary Figure S6. The reverse MR Analysis results are shown in Table 13.

Discussion

This study is the first to examine the causal relationship between 91 circulating inflammatory proteins and asthma and their phenotypes. A total of 30 potentially causal results were obtained, and after FDR correction, the occurrence of

Table 12 Effects of the Relationship Between Meaningful Circulating Inflammatory Proteins and Obesity Related Asthma in MR Analysis

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-FDR*	*P-Qtest*	*P-intercept*
FGF-5	Obesity related asthma	IVW	24	1.104	1.018	1.198	0.017	0.790	0.566	
		MR Egger	24	1.157	1.023	1.308	0.030		0.565	0.328
IL-17A	Obesity related asthma	IVW	13	1.235	1.021	1.493	0.030	0.900	0.943	
		MR Egger	13	1.232	0.760	1.996	0.416		0.910	0.992
PD-L1	Obesity related asthma	IVW	18	0.781	0.663	0.922	0.003	0.310	0.468	
		MR Egger	18	0.682	0.452	1.028	0.086		0.433	0.488

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR; Odds ratio.

Table 13 Effects of the Relationship Between Meaningful Suggestive for Obesity Related Asthma and Circulating Inflammatory Proteins in Reverse MR Analysis

Exposure	Outcome	Method	nsnp	OR	OR_lci95	OR_uci95	*P*	*P-Qtest*	*P-intercept*
Obesity related asthma	FGF-5	IVW	19	0.997	0.941	1.057	0.928	0.183	
		MR Egger	19	1.201	1.037	1.390	0.026	0.507	0.017
Obesity related asthma	IL-17A	IVW	19	1.059	0.997	1.124	0.062	0.185	
		MR Egger	19	1.197	1.015	1.412	0.048	0.261	0.138
Obesity related asthma	PD-L1	IVW	19	0.962	0.907	1.021	0.204	0.052	
		MR Egger	19	0.965	0.804	1.158	0.704	0.038	0.977

Abbreviations: nsnp, number of single nucleotide polymorphisms; OR, Odds ratio.

errors was reduced, and finally, two inflammatory protein result with a significant causal relationship was identified. Due to the limitations of clinical trial selection, there are no studies to explore the causal relationship between CST5 and non-allergic asthma, LIF-R and the onset of asthma in children. The results with potential causality, it is still groundbreaking and has potential value for future research.

The findings suggest that for asthma, elevated levels of IL-8, CD6, and TNFRSF9 may lead to an increased risk, and elevated levels of CD40 may lead to a decreased risk. Consistent with the results of Marc-Malovrh M, the elevation of IL-5 and IL-8 in phlegm eosinophils may stimulate airway remodeling and may be a useful non-invasive biomarker and therapeutic target for accelerating FEV1 decline in asthmatic patients.[18] Similarly, in the Matsuda S experiment on patients with first wheezing and confirmed asthma, serum IL-8, and IL-12 values at first wheezing were significantly higher than those in acute asthma or control groups.[19] Previous studies have shown that CD6 is related to the pathogenesis of autoimmune diseases.[20] Although no clinical or animal studies have been conducted to date on its association with asthma. However, it is presumed that CD6 is involved in the development and activation of T cells.[21] Therefore, CD6 may influence asthma development by affecting T cells. We have observed that TNFRSF9 levels are associated with an increased risk of asthma, but there are currently no studies to support this result, and further research is necessary to fully understand the role of this protein in maintaining health or promoting disease development.

The interaction between CD40 and its ligand CD40L controls humoral and cell-mediated immune responses, and CD40 expressed on connected airway epithelial cells can up-regulate the expression of inflammatory mediators.[22,23] In a test on the association of CD40 polymorphism with asthma risk and serum IgE level in a Korean population, the results showed that CD40 gene polymorphism regulates the expression of CD40 in B cells through translation, and has a genetic influence on the production of IgE in asthmatic patients.[24]

Genetically determined ARTN levels have a potential causal relationship with childhood asthma. In a case-control study that included 423 children with asthma and 414 non-asthmatic controls, 17 SNPS were significantly associated with asthma, of which 6 ARTN-related SNPS were still significantly associated with asthma after adjustment.[25] This is the same as our result. The IL18R1 gene is a strong candidate for asthma. CCL25, LIF-R, and TNF were all associated with reduced asthma in children. The research results of Stenstad H and Wurbel M. A fully prove that CCL25 may be involved in T cell development.[26,28] Different from the results of MR, the results of Sen Y. showed that the CCR9/CCL25

signaling pathway could interact with CD226 signaling to activate asthma NKT cells, leading to airway hyperreactivity and inflammation, which aggravated asthma.[29] IL-18 promotes airway inflammation and promotes auxiliary T-2 response.[30,31] Induces IgE, IL-4 and IL-13, and histamine release from basophils.[32,33] In earlier reports, IL-18 levels in blood and sputum were found to increase significantly during asthma attacks.[34] The results of the Zheng X study showed that the serum LIF levels in subjects with atopic asthma were higher than those in non-atopic subjects, and LIF links nerve and allergic inflammatory processes.[35] There is also evidence that LIF may also play an important role in regulating neuro-immune system interactions during acute inflammatory injury and subsequent healing and recovery processes. Therefore, LIF can be used as a mediator of bidirectional crosstalk between nervous tissue and the immune system.[36]

TNF is a potent pro-inflammatory cytokine that has been linked to asthma and atopic activity. Among them, TNF-α is involved in the development of allergic diseases, especially asthma and atopic dermatitis. It plays a dual role in the regulation of immune response, not only as a pro-inflammatory mediator to initiate strong inflammatory response, but also as an immunosuppressive mediator to inhibit the development of autoimmune diseases and tumorigenesis, and plays a crucial role in maintaining immune homeostasis by limiting the degree and duration of inflammatory processes.[37] Castro J showed elevated levels of TNF-α in airway biopsies and bronchoalveolar lavage fluid in asthmatic patients. However, no significant association was found between TNFB alleles and atopic properties.[38] There have been no clear studies on the relationship between CCL25, LIF-R, and TNF and childhood asthma in terms of age.

Observational and longitudinal studies have identified biomarkers that can distinguish patients with allergic asthma from healthy controls. Genetically predicted IL-2 levels are associated with an increased risk of allergic asthma. Kannan AK's[39] experimental results suggest that IL-2-induced T cell kinases regulate TH2-mediated allergic airway inflammation by inhibiting IFN-γin naive CD4+ T cells. Genetically determined levels of SCF, TRAIL, and TWEAK are potentially associated with reduced risk of allergic asthma. Interestingly, the study[40] results show that allergic asthma is characterized by elevated cytokine SCF, and measuring its expression in serum protein levels or mRNA levels in PBMC will be an important parameter in diagnosing allergic asthma. This is contrary to our results. Since there were no significant positive results for this inflammatory protein after FDR correction, more clinical studies are needed to confirm the causal relationship between this inflammatory protein and allergic asthma. TRAIL may promote or conversely address inflammation in asthma by inducing apoptosis in a variety of cells.[40] This is consistent with our results. McGrath EE's[41] findings showed that animals lacking TRAIL showed delayed neutrophil apoptosis and increased neutrophil inflammation. The administration of exogenous TRAIL restored the WT phenotype of TRAIL-deficient mice and, importantly, accelerated neutrophil apoptosis and neutrophil count reduction in WT mice. TWEAK binds to receptor factor-induced type 14 (Fn14) and is involved in a variety of pathological processes, including angiogenesis, cell proliferation and death, inflammation, and carcinogenesis.[42] Airway remodeling in asthma can accelerate HASMC cell proliferation and migration by activating the NF-κB pathway from the TWEAK/Fn14 axis.[43] TWEAK and TGF-β1 have a synergistic effect in epithelial-mesenchymal transition and may contribute to chronic airway change and remodeling.[44]

Our results are similar to those of previous studies. Levels of CCL19, CST5, and IL-7 may reflect the risk of non-allergic asthma. Genetically, CX3CL1, LAP TGF-beta-1, and ST1A1 levels were associated with a reduced risk of non-allergic asthma. Previous findings related to asthma or allergic asthma, but there was uncertainty about whether it was related to non-allergic asthma. Nakano K's[45] results show recombinant CCL19 increased the phosphorylation of STAT5 in mice with allergic asthma and induced the expression of TH2 cells and genes associated with the IL-2 signaling pathway. There is a clear causal relationship between IL-7 and asthma. Defects in the human and mouse IL-7 pathway lead to severe combined immune deficiency caused by lymphocytopenia.[46] However, due to the lack of specific studies on IL-7 and non-allergic asthma, a clear positive and negative causal relationship needs to be supported by other results. Similarly, studies have demonstrated that loss of CX3CR1 signaling unexpectedly leads to severe impairment of lung function. The CX3CL1/CX3CR1 axis preserves lung function during fungus-associated allergic airway inflammation through non-classical immunomodulatory mechanisms.[47] Unfortunately, there are no relevant studies on LAP TGF-beta-1 and ST1A1 levels and asthma, and it is impossible to know whether there is a causal relationship in clinical practice.

For eosinophilic asthma, only TNFB has been studied, but most of them are at the genetic level. Tumor necrosis factor (TNF; TNFA and TNFB) are major pro-inflammatory cytokines that are important in the pathogenesis of asthma.[48] A gene-level study from Japan showed that TNFA and TNFB genes encoding TNF-α and TNF-β, respectively, were associated with atopic asthma.[49] However, results from Egypt showed that TNFA-1031C >T and TNFA-308G >A polymorphisms were strongly associated with asthma risk ($p = 0.007$ and $p = 0.000$), but TNFB +252A>G polymorphisms were not ($p = 0.6$).[50]

For obesity-related asthma, FGF-5, IL-17A, and PD-L1 levels are causally related to it at the genetic level. Among them, the relationship between PD-L1 and obesity asthma was positive. The IL-17 axis is involved in the pathogenesis of ovalbumin-sensitized rat disease and is one of the therapeutic targets for obesity-related asthma subjects.[51] Unlike MR Analysis, PD-L1 has a bidirectional effect on the mechanism of asthma. PD-L1 can interact with PD-1 to activate Th2 and make it produce more IL-4, resulting in airway hyperreactivity. However, PD-L1 can also bind to PD-1 on the surface of immune cells (such as T cells) to promote the immunosuppressive effect of PD-1, such as reducing T cell proliferation, cytokine secretion, and cytotoxicity, and contributing to tissue homeostasis in the inflammatory response.[52] Unfortunately, the clinical correlation between FGF-5 and asthma is not high, and there is no specific study.

This study also has certain limitations: (1) Although some MR Results showed a potential causal relationship, it was never demonstrated in clinical or animal experiments, such as TNFRSF9, CXCL6, CST5, FGF-5 and SLAMF1. Therefore, whether this inflammatory factor has practical significance with asthma or its phenotype needs more research to clarify; (2) The experimental verification of the age level of disease is insufficient, and more studies are needed to confirm it; (3) Due to the fact that the population used is European, there may also be some limitations in the generalization of the results to other ethnic groups. (4) Since this study is a statistical analysis, it is not possible to explore the mechanism between various inflammatory factors and diseases, and more follow-up studies may be needed to clarify it.

In summary, this MR Analysis explored the causal relationship between 91 inflammatory factors and asthma and 5 phenotypes, providing new evidence for the demonstration of various inflammatory factors and asthma and their phenotypes, and verified previous studies to provide directions for future research.

Data Sharing Statement

The authors are prepared to share that all data used are from publicly available datasets, with disease data sourced from https://www.finngen.fi/fi, and complete protein GWAS summary statistics can be found at https://www.phpc.cam.ac.uk/ceu/proteins and in the EBI GWAS catalog (GCST90274758 - GCST90274848) for download. The above data have been included in the article/supplementary material, which can be found directly in the file "Supplementary material".

Ethics Approval

The data in this study were obtained from published studies, of which all data had been approved by the institutional review committee. The ethical application for this study was approved by the Medical Ethics Committee of Affiliated Hospital of Liaoning University of Traditional Chinese Medicine [ID: Y2023172CS(KT)-172-01].

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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