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ORIGINAL RESEARCH

Genetically Predicted t^6A (a tRNA-Derived Adenosine Modification) and Risk of Bullous Pemphigoid: A Two-Sample Mendelian Randomization Study

Lulu Yan, Dan Chen, Dehou Yu, Xiaoping Shen

Department of Dermatology and Sexually Transmitted Diseases, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, 550004, People's Republic of China

Correspondence: Xiaoping Shen, Email xiaopingpf@163.com

Purpose: Bullous pemphigoid (BP) is an autoimmune blistering disease linked to T-lymphocyte dysregulation. Adenosine deaminase (ADA) and adenosine metabolites modulate T-cell function, suggesting a potential role in BP pathogenesis. However, causal evidence from human genetic studies is lacking. This study aimed to investigate the potential causal associations of genetically predicted levels of ADA, ADA protein, and several adenosine metabolites with the risk of BP and its subtypes (mucous membrane pemphigoid [MMP] and other/unspecified pemphigoid [OUP]).

Patients and Methods: We conducted a two-sample Mendelian randomization (MR) study using summary statistics from large-scale genome-wide association studies (GWAS). Genetic instruments for ADA levels, ADA protein levels, 5-methylthioadenosine, N1-methyladenosine, N6-carbamoylthreonyladenosine (t^6A), and N6-succinyladenosine were selected. Outcome data for BP, MMP, and OUP were obtained from the FinnGen R12 release. The primary analysis used the inverse-variance weighted (IVW) method. Sensitivity analyses included MR-Egger, weighted median, weighted mode methods, Cochran's Q test, MR-Egger intercept test, MR-PRESSO, and leave-one-out analysis.

Results: Genetically predicted higher t^6A levels were significantly associated with a lower risk of BP (IVW OR: 0.37, 95% Confidence Interval [CI]: 0.21–0.66; $P < 0.001$; P_FDR < 0.001). This association was supported by the weighted median method (OR: 0.39, 95% CI: 0.18–0.86; $P = 0.02$). No significant evidence of heterogeneity or horizontal pleiotropy was found for this association. No causal associations were observed for other adenosine metabolites, ADA levels, or ADA protein levels with BP, MMP, or OUP after FDR correction.

Conclusion: This MR study suggests a potential causal association between higher t^6A levels and reduced risk of bullous pemphigoid. Further research is warranted to elucidate the underlying mechanisms and potential therapeutic implications.

Keywords: bullous pemphigoid, N6-carbamoylthreonyladenosine, adenosine deaminase, Mendelian randomization, causality, autoimmune skin diseases

Introduction

Bullous pemphigoid (BP) is the most common autoimmune subepidermal blistering disease, predominantly affecting the elderly population, and its incidence is reportedly increasing. The prevalence ranges from 0.21 to 7.63 cases per 100,000 people.[1,2] Clinically, BP is characterized by tense blisters on erythematous or urticarial plaques, often accompanied by intense pruritus.[3] The pathogenesis of BP is primarily mediated by autoantibodies, particularly IgG, which target two hemidesmosomal proteins, BP180 (type XVII collagen) and BP230. This leads to complement activation, inflammatory cell infiltration (including neutrophils, eosinophils, and lymphocytes), and subsequent dermo-epidermal separation.[4,5] While B cells are directly responsible for the production of pathogenic autoantibodies in BP, T lymphocytes, especially CD4+ T helper (Th)

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cells—including Th2 and T follicular helper (Tfh) subsets—are essential for providing help to B cells, orchestrating their differentiation and promoting class-switch recombination necessary for IgG autoantibody production.[6–8]

Adenosine deaminase (ADA) is a key enzyme in purine metabolism, catalyzing the irreversible deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively.[9] ADA activity is crucial for lymphocyte proliferation, differentiation, and function, particularly in T cells; its deficiency leads to severe combined immunodeficiency.[10] Both ADA levels and ADA protein levels can be measured, and while related, their genetic regulation may differ, with protein levels more directly reflecting enzyme abundance. ADA activity is also considered an indirect marker of T cell activation.[11] Adenosine, regulated by ADA, is a potent endogenous immunomodulator, generally exerting anti-inflammatory effects via adenosine receptors (eg., A2A receptor) on immune cells, including T-cells.[12,13] The interaction of ADA with molecules such as dipeptidyl peptidase-4 (DPP4), which can also influence T cell biology, has been implicated in BP pathogenesis, suggesting complex regulatory networks within the immune system.[14] In addition, various adenosine metabolites, such as t^6A, a modified nucleoside found in tRNA, exist, although their specific roles in systemic immune regulation are less well characterized.[15] Given that B cell-driven antibody production in BP fundamentally depends on T cell help, particularly from Tfh and Th2 cells, and considering the modulatory effects of the ADA–adenosine axis on T cell function, it is plausible that variations in ADA levels, ADA protein levels, or specific adenosine metabolites might influence susceptibility to BP.

To date, no observational studies have directly linked t^6A or adenosine deaminase (ADA) levels with the risk of developing bullous pemphigoid. Observational studies remain subject to confounding from comorbidities, aging, and medication exposures, as well as to reverse causality, limiting their ability to establish causal inferences. Mendelian randomization (MR) uses genetic variants as instrumental variables (IVs) for an exposure to assess its causal effect on an outcome, thereby minimizing bias from confounding and reverse causation.[16,17] By leveraging randomly allocated genetic variants, MR can mimic some aspects of a randomized controlled trial.

This study aimed to employ a two-sample MR approach to investigate the potential causal relationships between genetically predicted levels of ADA, ADA protein, and several adenosine metabolites (5-methylthioadenosine, N1-methyladenosine, N6-carbamoylthreonyladenosine, and N6-succinyladenosine) and the risk of BP and its clinical subtypes (mucous membrane pemphigoid [MMP] and other/unspecified pemphigoid).

Material and Methods

Study Design

This two-sample MR study utilized summary-level data from publicly available genome-wide association studies (GWAS). The study adhered to the three core assumptions of MR: (i) the genetic IVs are robustly associated with the exposure; (ii) the IVs are not associated with confounders of the exposure-outcome relationship; and (iii) the IVs affect the outcome exclusively through the exposure (Figure 1).[18] This study was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) guidelines (Supplementary Table S1).[19]

This study uses only publicly available, anonymized summary-level GWAS data and does not involve identifiable human subjects. According to Article 32 (Items 1 and 2) of the *Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects* (China, 2023), it is exempt from ethical review. All original studies had obtained relevant ethics approval and informed consent.

Data Sources

Summary statistics for six exposures—ADA levels, ADA protein levels, 5-methylthioadenosine levels, N1-methyladenosine levels, t^6A levels, and N6-succinyladenosine levels—were obtained from published GWAS or publicly available databases.[20–22]

Outcome data for BP, mucous membrane pemphigoid (MMP), and other/unspecified pemphigoid (OUP) were obtained from the FinnGen consortium R12 release (https://r12.finngen.fi/), which comprises individuals of Finnish

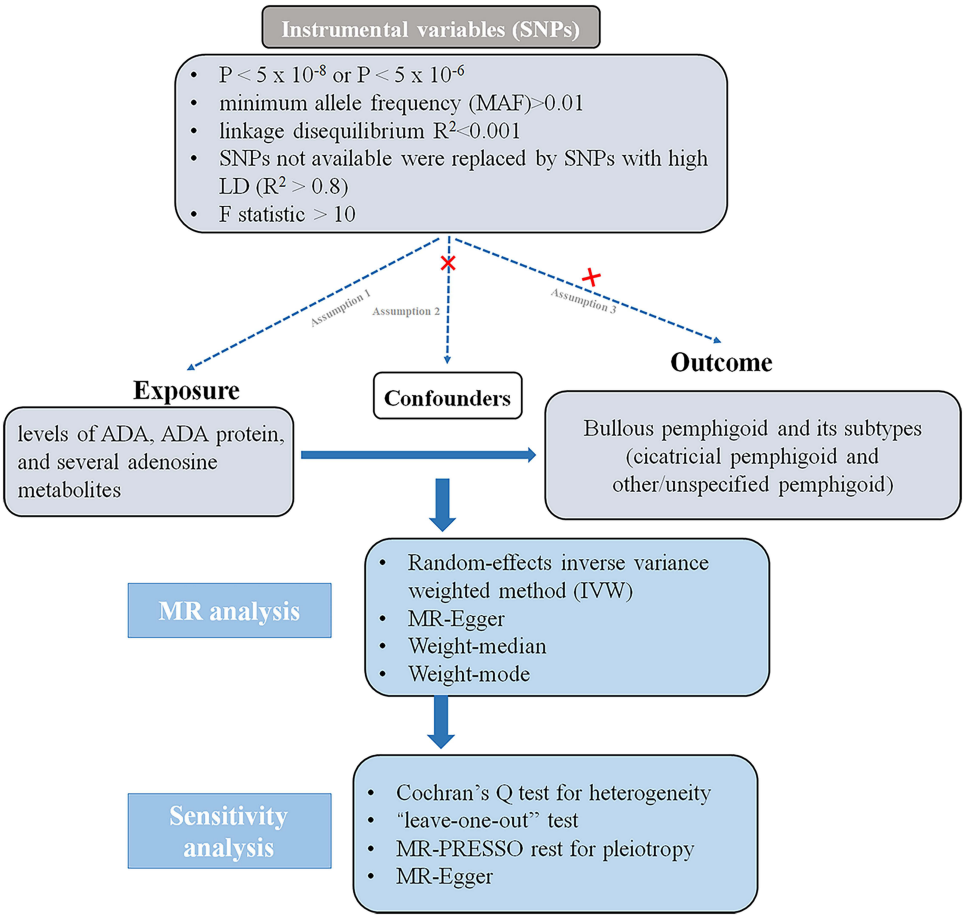

Figure 1 Study design flowchart of the two-sample Mendelian randomization analysis. The flow diagram illustrates the systematic process of the MR study, including instrumental variable selection, the three core MR assumptions, and the statistical methods employed. The dashed lines represent the three fundamental MR assumptions: Assumption 1 (relevance) indicates that genetic variants are robustly associated with the exposure; Assumption 2 (Independence) and Assumption 3 (exclusion restriction) are indicated by dashed lines with a red "X" symbol, signifying that the genetic variants must not be associated with confounders and must influence the outcome only via the exposure, respectively.

Abbreviations: ADA, adenosine deaminase; SNP, single-nucleotide polymorphism; LD, linkage disequilibrium; MAF, minor allele frequency; IVW, inverse variance weighted; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier.

(European) ancestry (Supplementary Table S2). Outcomes were defined using ICD-10 codes: L12.0 for BP, L12.1 for MMP, and L12.8 or L12.9 for OUP. The OUP group is defined based on ICD coding and typically includes cases that do not fulfill the diagnostic criteria for classic BP or MMP; thus, it represents a heterogeneous category of pemphigoid cases.

All exposure GWAS were conducted in individuals of European ancestry. Importantly, there was no sample overlap between the exposure GWAS datasets and the outcome data from the FinnGen R12 release, thus minimizing the potential for bias due to overlapping participants.

Instrumental Variable Selection

We applied rigorous criteria to select single nucleotide polymorphisms (SNPs) as IVs. For most exposures, SNPs were included if they were associated at genome-wide significance ($P < 5 \times 10^{-8}$), but for ADA levels and t^6A levels, the threshold was relaxed to $P < 5 \times 10^{-6}$ to ensure an adequate number of instruments, a common strategy in MR studies with limited IVs. SNPs with minor allele frequency (MAF) > 0.01 were retained, and linkage disequilibrium (LD) clumping was performed ($R^2 < 0.001$, window = 10,000 kb) using the 1000 Genomes Project European reference panel to ensure IV independence.[23–25] When an IV was not available in the outcome GWAS,

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a proxy SNP in high LD ($R^2 > 0.8$) was used. Instrument strength was assessed using the F-statistic, and only strong IVs ($F > 10$) were included to minimize weak instrument bias.[26] Harmonization of effect alleles between exposure and outcome datasets was performed to ensure alignment. Palindromic SNPs with intermediate allele frequencies, where strand orientation could not be confidently determined, were excluded.

MR and Statistical Analysis

The primary MR analysis was performed using the random-effects inverse-variance weighted (IVW) method to estimate the causal effect.[27] Odds ratios (ORs) and 95% confidence intervals (CIs) were reported per genetically predicted unit increase in exposure. To assess the robustness of findings and test for potential violations of MR assumptions, sensitivity analyses were conducted, including MR-Egger regression, weighted median estimator (WME), weighted mode estimator (WMBE),[28,29] Cochran's Q test for heterogeneity,[30] the MR-Egger regression intercept test, MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) test, and leave-one-out analysis.[31] All analyses were performed using the "TwoSampleMR" and "MRPRESSO" packages in R software (version 4.0.5). Statistical significance for the primary IVW estimates was defined as a P-value corrected for multiple testing using the False Discovery Rate (FDR) procedure ($P\_FDR < 0.05$).

Results

Instrumental Variable Characteristics

Following the selection and harmonization process, we successfully identified valid IVs for all six exposures. The final number of IVs used for the analysis of adenosine deaminase levels, ADA protein levels, 5-methylthioadenosine, N1-methyladenosine, N6-carbamoylthreonyladenosine (t^6A), and N6-succinyladenosine were 24, 14, 11, 6, 7, and 3, respectively. All selected instruments were strong, with mean F-statistics ranging from 23.18 to 218.92, well above the threshold of 10, indicating that weak instrument bias was unlikely. Detailed characteristics of all IVs, including information on proxy SNPs and gene annotations, are provided in Supplementary Table S3.

Mendelian Randomization Analysis

Our primary finding reveals a significant causal association between genetically predicted higher levels of N6-carbamoylthreonyladenosine (t^6A) and a reduced risk of BP (Table 1). The inverse-variance weighted (IVW) analysis showed that genetically predicted t^6A levels was associated with a 63% lower risk of BP (OR: 0.37, 95% CI: 0.21–0.66, $P < 0.001$, $P\_FDR < 0.001$). This protective association was further supported by the weighted median method (OR: 0.39, 95% CI: 0.18–0.86, nominal $P = 0.020$), and directionally consistent results were observed across all MR methods in the forest plot (Figure 2A). The scatter plot visually confirmed this protective trend, with most SNPs aligning along a negative slope (Figure 2B). A comprehensive set of sensitivity analyses confirmed the robustness of this result. We found no evidence of significant heterogeneity (Cochran's Q for IVW $P = 0.48$) or directional horizontal pleiotropy (MR-Egger intercept $P = 0.66$) (Table 2). The MR-PRESSO test detected no significant outliers ($P = 0.526$) (Table 3), the funnel plot appeared largely symmetrical, suggesting no substantial pleiotropy (Figure 2C), and the leave-one-out analysis demonstrated that the association was not driven by any single influential SNP (Figure 2D).

In contrast, after correcting for multiple testing, we found no statistically significant causal associations between genetically predicted levels of ADA, ADA protein, 5-methylthioadenosine, N1-methyladenosine, or N6-succinyladenosine and the risk of BP. Similarly, our exploratory analyses for the rarer subtypes, MMP (70 cases) and other/unspecified pemphigoid (OUP, 274 cases), did not yield any significant causal associations for any of the six exposures. These null findings, particularly for MMP and OUP, must be interpreted with caution due to severely limited statistical power and a high likelihood of Type II errors (Tables 1–3).

Table 1 Mendelian Randomization Estimates for the Causal Association of Genetically Predicted Adenosine-Related Exposures with the Risk of Bullous Pemphigoid

Exposure	Outcome	N.SNPs	Methods	OR (95% CI)	P	P_FDR
Adenosine Deaminase levels	Bullous pemphigoid	24	Inverse variance weighted	0.9814 (0.7823–1.2312)	0.87	0.92
Adenosine Deaminase levels		24	MR Egger	1.0444 (0.7681–1.4203)	0.78	0.9
Adenosine Deaminase levels		24	Weighted median	0.9274 (0.739–1.1638)	0.52	0.9
Adenosine Deaminase levels		24	Weighted mode	0.9385 (0.7452–1.182)	0.59	0.9
ADA protein levels	Bullous pemphigoid	14	Inverse variance weighted	1.0615 (0.7804–1.4439)	0.7	0.9
ADA protein levels		14	MR Egger	1.2238 (0.7696–1.9461)	0.41	0.9
ADA protein levels		14	Weighted median	1.1273 (0.7627–1.6661)	0.55	0.9
ADA protein levels		14	Weighted mode	1.0604 (0.7699–1.4606)	0.73	0.9
5-methylthioadenosine levels	Bullous pemphigoid	11	Inverse variance weighted	0.7543 (0.3284–1.7323)	0.51	0.9
5-methylthioadenosine levels		11	MR Egger	0.1168 (0.0099–1.3738)	0.12	0.9
5-methylthioadenosine levels		11	Weighted median	0.6336 (0.2428–1.6535)	0.35	0.9
5-methylthioadenosine levels		11	Weighted mode	0.5617 (0.1762–1.7909)	0.35	0.9
N1-methyladenosine levels	Bullous pemphigoid	6	Inverse variance weighted	0.69 (0.359–1.3262)	0.27	0.9
N1-methyladenosine levels		6	MR Egger	0.7366 (0.2586–2.0984)	0.6	0.9
N1-methyladenosine levels		6	Weighted median	0.9068 (0.4439–1.8527)	0.79	0.9
N1-methyladenosine levels		6	Weighted mode	0.938 (0.4328–2.0329)	0.88	0.92
N6-carbamoylthreonyladenosine levels	Bullous pemphigoid	7	Inverse variance weighted	0.3703 (0.2072–0.6619)	<0.001	<0.001
N6-carbamoylthreonyladenosine levels		7	MR Egger	0.6551 (0.0538–7.9804)	0.75	0.9
N6-carbamoylthreonyladenosine levels		7	Weighted median	0.3893 (0.1759–0.8617)	0.02	0.24
N6-carbamoylthreonyladenosine levels		7	Weighted mode	0.3994 (0.1295–1.2313)	0.16	0.9
N6-succinyladenosine levels	Bullous pemphigoid	3	Inverse variance weighted	0.7195 (0.1715–3.018)	0.65	0.9
N6-succinyladenosine levels		3	MR Egger	0.0265 (0–750.9085)	0.61	0.9
N6-succinyladenosine levels		3	Weighted median	0.8029 (0.2439–2.643)	0.72	0.9
N6-succinyladenosine levels		3	Weighted mode	0.9973 (0.3073–3.2364)	1	1
Adenosine Deaminase levels	Mucous membrane pemphigoid	24	Inverse variance weighted	1.2524 (0.7029–2.2317)	0.45	0.93
Adenosine Deaminase levels		24	MR Egger	0.8453 (0.3892–1.8358)	0.68	0.93
Adenosine Deaminase levels		24	Weighted median	0.8661 (0.4246–1.7665)	0.69	0.93
Adenosine Deaminase levels		24	Weighted mode	0.8979 (0.453–1.7799)	0.76	0.93
ADA protein levels	Mucous membrane pemphigoid	14	Inverse variance weighted	0.804 (0.3069–2.1064)	0.66	0.93
ADA protein levels		14	MR Egger	0.8688 (0.2032–3.7144)	0.85	0.93
ADA protein levels		14	Weighted median	0.9386 (0.2907–3.0304)	0.92	0.96
ADA protein levels		14	Weighted mode	0.9016 (0.3256–2.4964)	0.85	0.93
5-methylthioadenosine levels	Mucous membrane pemphigoid	11	Inverse variance weighted	0.5733 (0.0624–5.2643)	0.62	0.93
5-methylthioadenosine levels		11	MR Egger	8.6215 (0.0076–9769.2426)	0.56	0.93
5-methylthioadenosine levels		11	Weighted median	1.6539 (0.0872–31.3596)	0.74	0.93
5-methylthioadenosine levels		11	Weighted mode	1.6875 (0.0483–58.9889)	0.78	0.93

(*Continued*)

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Table 1 (Continued).

Exposure	Outcome	N.SNPs	Methods	OR (95% CI)	P	P_FDR
N1-methyladenosine levels	Mucous membrane pemphigoid	6	Inverse variance weighted	1.7222 (0.264–11.236)	0.57	0.93
N1-methyladenosine levels		6	MR Egger	0.231 (0.0162–3.3015)	0.34	0.93
N1-methyladenosine levels		6	Weighted median	0.9419 (0.0893–9.9359)	0.96	0.96
N1-methyladenosine levels		6	Weighted mode	0.69 (0.057–8.3466)	0.78	0.93
N6-carbamoylthreonyladenosine levels	Mucous membrane pemphigoid	7	Inverse variance weighted	0.1818 (0.0295–1.1216)	0.07	0.64
N6-carbamoylthreonyladenosine levels		7	MR Egger	2e-04 (0–0.3453)	0.08	0.64
N6-carbamoylthreonyladenosine levels		7	Weighted median	0.1215 (0.0119–1.2425)	0.08	0.64
N6-carbamoylthreonyladenosine levels		7	Weighted mode	0.0783 (0.0022–2.847)	0.21	0.93
N6-succinyladenosine levels	Mucous membrane pemphigoid	3	Inverse variance weighted	0.3849 (0.015–9.8751)	0.56	0.93
N6-succinyladenosine levels		3	MR Egger	9.2886 (0–64,095,786.6032)	0.83	0.93
N6-succinyladenosine levels		3	Weighted median	0.4155 (0.012–14.3956)	0.63	0.93
N6-succinyladenosine levels		3	Weighted mode	0.4009 (0.0096–16.825)	0.68	0.93
Adenosine Deaminase levels	Other and unspecified pemphigoid	24	Inverse variance weighted	1.0992 (0.8189–1.4754)	0.53	0.94
Adenosine Deaminase levels		24	MR Egger	1.175 (0.7919–1.7433)	0.43	0.94
Adenosine Deaminase levels		24	Weighted median	1.1151 (0.7849–1.5843)	0.54	0.94
Adenosine Deaminase levels		24	Weighted mode	1.0855 (0.7624–1.5457)	0.65	0.94
ADA protein levels	Other and unspecified pemphigoid	14	Inverse variance weighted	1.0851 (0.6644–1.7721)	0.74	0.94
ADA protein levels		14	MR Egger	1.6779 (0.8011–3.5144)	0.2	0.94
ADA protein levels		14	Weighted median	1.1362 (0.6255–2.0641)	0.68	0.94
ADA protein levels		14	Weighted mode	1.2085 (0.7395–1.9752)	0.46	0.94
5-methylthioadenosine levels	Other and unspecified pemphigoid	11	Inverse variance weighted	0.5093 (0.1571–1.6511)	0.26	0.94
5-methylthioadenosine levels		11	MR Egger	0.6122 (0.0119–31.4077)	0.81	0.94
5-methylthioadenosine levels		11	Weighted median	0.632 (0.1211–3.2983)	0.59	0.94
5-methylthioadenosine levels		11	Weighted mode	1.398 (0.1559–12.5365)	0.77	0.94
N1-methyladenosine levels	Other and unspecified pemphigoid	6	Inverse variance weighted	0.7335 (0.2861–1.8808)	0.52	0.94
N1-methyladenosine levels		6	MR Egger	0.8553 (0.2254–3.2462)	0.83	0.94
N1-methyladenosine levels		6	Weighted median	0.7771 (0.2478–2.4372)	0.67	0.94
N1-methyladenosine levels		6	Weighted mode	0.8148 (0.2575–2.5779)	0.74	0.94
N6-carbamoylthreonyladenosine levels	Other and unspecified pemphigoid	7	Inverse variance weighted	0.6486 (0.1669–2.5208)	0.53	0.94
N6-carbamoylthreonyladenosine levels		7	MR Egger	0.2075 (5e-04-95.6271)	0.64	0.94
N6-carbamoylthreonyladenosine levels		7	Weighted median	0.4836 (0.1251–1.8703)	0.29	0.94
N6-carbamoylthreonyladenosine levels		7	Weighted mode	0.2411 (0.0178–3.2622)	0.33	0.94
N6-succinyladenosine levels	Other and unspecified pemphigoid	3	Inverse variance weighted	1.0823 (0.1867–6.2724)	0.93	0.94
N6-succinyladenosine levels		3	MR Egger	5.9529 (1e-04-364,471.8197)	0.8	0.94
N6-succinyladenosine levels		3	Weighted median	0.9338 (0.1345–6.4834)	0.94	0.94
N6-succinyladenosine levels		3	Weighted mode	0.8502 (0.1225–5.9033)	0.88	0.94

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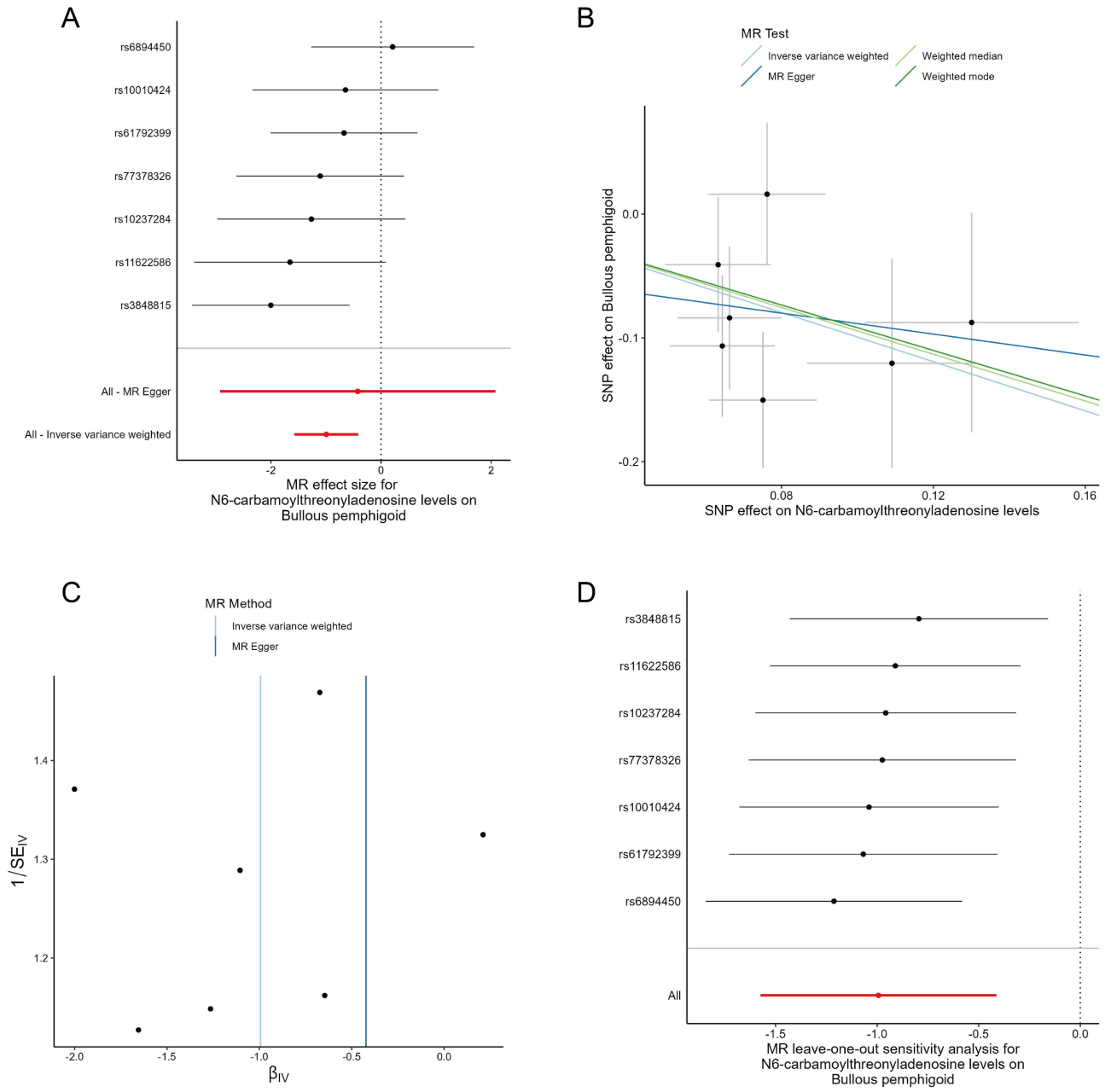

Figure 2 Mendelian randomization plots for the association of genetically predicted N6-carbamoylthreonyladenosine levels with the risk of bullous pemphigoid. (**A**) Forest plot showing the causal estimates from different Mendelian randomization (MR) methods. (**B**) Scatter plot illustrating the relationship between the SNP effects on N6-carbamoylthreonyladenosine levels (x-axis) and their effects on bullous pemphigoid (y-axis). (**C**) Funnel plot for assessing heterogeneity and potential asymmetry. (**D**) Leave-one-out sensitivity analysis to evaluate the influence of individual SNPs on the overall causal estimate.

Discussion

This two-sample MR study investigated the causal relationships between genetically predicted levels of ADA, ADA protein, and several adenosine metabolites with the risk of BP and its subtypes. Our primary finding indicates that genetically predicted higher levels of t^6A are causally associated with a significantly reduced risk of developing bullous pemphigoid. This association, representing approximately a 63% reduction in BP risk in t^6A levels, was robust across the primary IVW analysis and nominally supported by the weighted median method, with no evidence of significant heterogeneity or horizontal pleiotropy. Conversely, we found no strong evidence for causal associations between genetically predicted levels of ADA, ADA protein, 5-methylthioadenosine, N1-methyladenosine, or N6-

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Table 2 Sensitivity Analyses (Cochran's Q for Heterogeneity and MR-Egger Intercept for Pleiotropy) for All Mendelian Randomization Associations

Exposure	Outcome	Heterogeneity		Pleiotropy	
		Q Statistic (IVW)	*P* value	MR-Egger Intercept	*P* value
Adenosine Deaminase levels	Bullous pemphigoid	34.89	0.05	−0.024	0.56
ADA protein levels		9.49	0.73	−0.023	0.44
5-methylthioadenosine levels		14.21	0.16	0.404	0.15
N1-methyladenosine levels		6.45	0.26	−0.016	0.87
N6-carbamoylthreonyladenosine levels		5.51	0.48	−0.046	0.66
N6-succinyladenosine levels	Mucous membrane pemphigoid	3.52	0.17	0.909	0.64
Adenosine Deaminase levels		12.9	0.95	0.154	0.15
ADA protein levels		9.69	0.72	−0.013	0.89
5-methylthioadenosine levels		6.41	0.78	−0.577	0.45
N1-methyladenosine levels		4.54	0.47	0.476	0.11
N6-carbamoylthreonyladenosine levels		5.31	0.5	0.564	0.12
N6-succinyladenosine levels		0.3	0.86	−0.911	0.76
Adenosine Deaminase levels	Other and unspecified pemphigoid	16.06	0.85	−0.026	0.62
ADA protein levels		9.18	0.76	−0.072	0.15
5-methylthioadenosine levels		10.82	0.37	−0.04	0.93
N1-methyladenosine levels		3.46	0.63	−0.037	0.77
N6-carbamoylthreonyladenosine levels		12.87	0.05	0.092	0.72
N6-succinyladenosine levels		0.99	0.61	−0.467	0.81

Table 3 MR-PRESSO Global Test Results for Outlier Detection in All Mendelian Randomization Analyses

Exposure	Outcome	Raw		Outlier Corrected		Global P	Number of Outliers	Distortion P
		OR (CI%)	*P*	OR (CI%)	*P*			
Adenosine Deaminase levels.1	Bullous pemphigoid	0.98 (0.78–1.23)	0.87	NA	NA	0.115	NA	NA
ADA protein levels.1		1.06 (0.82–1.38)	0.66	NA	NA	0.724	NA	NA
5-methylthioadenosine levels.1		0.75 (0.33–1.73)	0.52	NA	NA	0.179	NA	NA
N1-methyladenosine levels.1		0.69 (0.36–1.33)	0.32	NA	NA	0.345	NA	NA
N6-carbamoylthreonyladenosine levels.1		0.37 (0.21–0.65)	0.01	NA	NA	0.526	NA	NA
Adenosine Deaminase levels.1	Mucous membrane pemphigoid	1.25 (0.81–1.93)	0.32	NA	NA	0.81	NA	NA
ADA protein levels.1		0.8 (0.35–1.85)	0.62	NA	NA	0.74	NA	NA
5-methylthioadenosine levels.1		0.57 (0.1–3.38)	0.55	NA	NA	0.811	NA	NA
N1-methyladenosine levels.1		1.72 (0.29–10.29)	0.58	NA	NA	0.488	NA	NA
N6-carbamoylthreonyladenosine levels.1		0.18 (0.03–1.01)	0.1	NA	NA	0.548	NA	NA
Adenosine Deaminase levels.1	Other and unspecified pemphigoid	1.1 (0.86–1.41)	0.46	NA	NA	0.879	NA	NA
ADA protein levels.1		1.09 (0.72–1.64)	0.7	NA	NA	0.81	NA	NA
5-methylthioadenosine levels.1		0.51 (0.16–1.65)	0.29	NA	NA	0.368	NA	NA
N1-methyladenosine levels.1		0.73 (0.34–1.61)	0.47	NA	NA	0.698	NA	NA
N6-carbamoylthreonyladenosine levels.1		0.65 (0.17–2.52)	0.55	NA	NA	0.074	NA	NA

succinyladenosine and any of the pemphigoid outcomes investigated after accounting for multiple testing. However, these null findings must be interpreted with substantial caution, as the limited sample sizes of the exposure datasets likely resulted in insufficient statistical power, potentially masking true causal relationships.

The protective association observed for t^6A with BP is a novel finding. t^6A is a universally conserved hypermodified nucleoside found at position 37 (A37), 3'-adjacent to the anticodon, in tRNAs decoding ANN codons (where N is any nucleotide).[15,32] This modification is crucial for accurate and efficient protein translation by stabilizing codon-anticodon interactions and preventing frameshifting errors.[33] The pivotal question arising from our study is: how does a molecule primarily involved in the fidelity of protein synthesis exert a protective effect against a T-cell-driven autoimmune disease like BP? The GWAS for t^6A levels identified SNPs in key genes, including TNIK (rs61792399), providing a crucial genetic link to a specific biological pathway. TNIK acts as a downstream signaling molecule for tumor necrosis factor (TNF) superfamily receptors (such as CD27) and is an essential regulator of CD8+ T-cell fate decisions during an immune response.[34] Based on these genetic and bioinformatic observations, we hypothesize that higher t^6A levels might ensure the high-fidelity translation of TNIK, leading to a more controlled, memory-oriented T-cell response that reduces autoimmune-driven inflammation and lowers susceptibility to BP.

While the direct systemic immunological role of circulating t^6A levels is not well-defined, dysregulation of tRNA modifications, including t^6A, has been linked to various cellular stress responses, mitochondrial dysfunction, and diseases including cancer and neurodevelopmental disorders.[35] For instance, impaired t^6A modification can lead to proteotoxic stress,[36] which could exacerbate inflammatory responses. Circulating levels of modified nucleosides like t^6A can reflect the overall tRNA turnover and modification status within cells,[37] potentially integrating signals from various tissues, including immune cells.[38] Adenosine itself, the parent molecule, generally exerts immunosuppressive effects, particularly via the A2A receptor on T-cells, reducing inflammation and promoting Treg function.[12,13] It remains unclear whether t^6A or its metabolic derivatives directly influence immune cell function in humans; future experimental studies are needed to address this possibility.

The null findings for ADA level, ADA protein levels and other adenosine metabolites with BP risk are somewhat surprising, given ADA's established role in T-cell function. It's possible that systemic ADA levels, as captured by these GWAS, do not adequately reflect the local ADA activity within the skin microenvironment or specific lymphocyte subsets critical for BP pathogenesis. Patel et al (2021) reviewed the role of DPP4, an enzyme interacting with ADA and influencing T-cell biology, in various cutaneous diseases including BP, suggesting complex interactions within this pathway.[14] In addition, from a statistical perspective, these null results are not unexpected given the small sample sizes of the available exposure GWAS, which significantly limit statistical power and increase the risk of Type II errors.

This study has several strengths. The MR design minimizes confounding and reverse causality. We utilized large-scale GWAS data for outcomes, enhancing statistical power for BP. Comprehensive sensitivity analyses were performed, largely supporting the validity of our main finding for t^6A. The F-statistics for all IVs were well above 10, reducing the likelihood of weak instrument bias.

Nevertheless, several limitations must be acknowledged. First, the GWAS datasets for several adenosine derivatives relied on relatively small sample sizes. Although F-statistics indicated sufficient instrument strength, the limited precision of SNP-exposure estimates restricts the statistical power of the MR analysis and may bias estimates towards the null. Consequently, the null findings for exposures such as ADA should be interpreted with caution due to the potential for Type II errors, whereas the significant association identified for t^6A despite these limitations is particularly notable Second, all genetic data were derived from individuals of European ancestry, with outcomes specifically from the Finnish population, which restricts the generalizability of our findings to other ethnic groups. Third, while sensitivity analyses showed no strong evidence of directional pleiotropy for the t^6A-BP association, unmeasured or balanced horizontal pleiotropy cannot be entirely excluded. Additionally, the capacity of methods like MR-Egger regression to detect pleiotropy is limited when the number of instrumental variables is small. Finally, MR estimates reflect the effect of lifelong, genetically determined exposure levels, which may not perfectly mirror the impact of acquired variations or therapeutic interventions later in life.

Conclusions

In summary, our results provide preliminary, hypothesis-generating evidence that genetically higher t^6A levels may reduce BP risk. However, given the limitations of exposure GWAS sample size, the single outcome dataset, lack of external replication, and untested mechanisms, these findings should be interpreted with caution. Larger, multi-ethnic

studies and functional research are needed to confirm these observations and to explore the potential immunological roles of t^6A and ADA in BP pathogenesis.

Data Sharing Statement

All data generated or analysed during this study are included in this published article.

Ethics Approval and Informed Consent

This study is based solely on publicly available, anonymized summary-level data from GWAS. According to Items 1 and 2 of Article 32 of the **Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects** (February 18, 2023, China), such research is exempt from ethics review and approval. All original studies contributing data had received appropriate ethics approval and participant written informed consent.

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

The authors declare that they have no competing interests in this work.

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