ISSN: 2754-6667

Journal of Critical Care & Emergency Medicine

SCIENTIFIC Research and Community

Review Article

Open Access

Epigenetic Modulation of Type 2 Immune Responses in Allergic Disorders: A Longitudinal Systems Biology Approach

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ABSTRACT

Background: Allergic disorders are among the fastest-growing chronic inflammatory diseases worldwide, affecting more than one billion individuals. Although genetic susceptibility contributes to disease development, it cannot fully explain the increasing prevalence of allergic conditions. Epigenetic mechanisms, including DNA methylation, histone modification, chromatin remodeling, and non-coding RNA regulation, have emerged as key mediators linking environmental exposures to dysregulated Type 2 immune responses.

Objective: This study investigated longitudinal epigenetic remodeling associated with Type 2 immune responses in allergic disorders using an integrated multi-omics systems biology approach and identified epigenetic signatures associated with disease progression and therapeutic responsiveness.

Methods: A multicenter longitudinal study was conducted between April 2023 and October 2025 in tertiary hospitals across Indonesia. Five hundred adults with clinically confirmed allergic disorders were followed for 24 months. Genome-wide DNA methylation, histone acetylation, transcriptomic profiling, and Type 2 inflammatory biomarkers, including IL-4, IL-5, IL-13, eosinophils, and total IgE, were analyzed. Longitudinal mixed-effects regression and systems biology analyses evaluated temporal associations between epigenetic alterations and immune dysregulation.

Results: Significant hypomethylation of **IL4** and **IL13** promoters was associated with increased cytokine expression ($\beta = -0.41$, $p < 0.001$). Enhanced H3K27 acetylation correlated with increased chromatin accessibility and Type 2 cytokine transcription. Greater epigenetic remodeling was associated with higher disease severity, recurrent exacerbations, delayed therapeutic responses, and persistent eosinophilic inflammation.

Conclusion: Longitudinal epigenetic remodeling is a key regulator of Type 2 immunity and may support biomarker discovery and precision medicine for allergic disorders.

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Received: October 10, 2026; **Accepted:** October 21, 2026; **Published:** October 26, 2026

Keywords: Epigenetics, DNA Methylation, Histone Modification, Type 2 Immunity, Allergic Disorders

Introduction

Allergic disorders have become one of the most significant global public health challenges of the twenty-first century. Their prevalence has increased steadily across both developed and developing countries, affecting individuals throughout the lifespan and contributing substantially to healthcare expenditures, reduced quality of life, work absenteeism, and premature disability. The World Health Organization estimates that allergic diseases continue to expand globally due to rapid urbanization, environmental pollution, climate change, alterations in microbial exposure, dietary transitions, and complex interactions between host genetics and environmental factors. Asthma alone affects more than 260 million individuals worldwide and remains responsible for hundreds of thousands of deaths annually, while allergic rhinitis and atopic dermatitis collectively affect nearly one-third of the global population [1,2].

The immunopathogenesis of allergic diseases is predominantly driven by dysregulated Type 2 immune responses orchestrated by T helper type 2 (Th2) lymphocytes, group 2 innate lymphoid cells (ILC2s), eosinophils, mast cells, basophils, and alternatively activated macrophages. These immune cells produce characteristic cytokines, including interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-9 (IL-9), interleukin-13 (IL-13), and interleukin-31 (IL-31), which collectively stimulate immunoglobulin E (IgE) production, eosinophilic recruitment, mucus hypersecretion, airway hyperresponsiveness, tissue remodeling, and chronic inflammation [3,4]. Persistent activation of these pathways contributes not only to disease onset but also to disease progression, recurrent exacerbations, corticosteroid resistance, and long-term structural changes within affected tissues.

Despite considerable advances in genome-wide association studies, identified genetic polymorphisms explain only a relatively small proportion of disease susceptibility. The rapid increase in allergic disease prevalence over recent decades cannot be adequately explained by alterations in genomic sequences

because human genetic evolution occurs over substantially longer timescales. Consequently, growing attention has shifted toward epigenetic regulation as a plausible biological mechanism linking environmental exposures with altered immune function [5,6].

Epigenetics refers to heritable yet reversible modifications regulating gene expression without altering the underlying DNA sequence. These regulatory processes include DNA methylation, post-translational histone modifications, chromatin remodeling, nucleosome positioning, and multiple classes of regulatory non-coding RNAs. Together, these mechanisms determine chromatin accessibility and transcriptional activity, thereby influencing immune-cell differentiation, inflammatory signaling, and adaptive immune memory [7,8].

DNA methylation remains among the most extensively investigated epigenetic mechanisms in allergy research. Methylation of cytosine residues within CpG islands located in promoter regions generally suppresses gene transcription by reducing transcription factor accessibility. Conversely, hypomethylation facilitates active transcription and may amplify inflammatory cytokine production. Several investigations have demonstrated that hypomethylation of IL4, IL13, and GATA3 promoter regions correlates strongly with enhanced Type 2 inflammation, elevated serum IgE concentrations, eosinophilia, and increased asthma severity [9-11].

Histone modifications represent another fundamental layer of epigenetic regulation. Histone acetyltransferases increase chromatin accessibility through acetylation of lysine residues, whereas histone deacetylases generally induce chromatin condensation and transcriptional repression. Histone methylation, phosphorylation, ubiquitination, and SUMOylation further contribute to dynamic regulation of inflammatory genes. In allergic diseases, increased histone acetylation has been consistently associated with activation of cytokine genes involved in Type 2 inflammation, whereas dysregulated histone deacetylase activity may contribute to corticosteroid resistance observed in severe asthma [12,13].

Recent advances in high-throughput sequencing technologies have revolutionized understanding of immune regulation by enabling comprehensive interrogation of the epigenome, transcriptome, proteome, metabolome, and cellular interactome simultaneously. These multi-omics technologies have generated unprecedented opportunities to investigate molecular mechanisms underlying chronic inflammatory diseases through integrated systems biology approaches. Rather than evaluating isolated molecular pathways, systems biology conceptualizes immune regulation as a dynamic and interconnected biological network involving thousands of interacting genes, proteins, metabolites, and signaling cascades [14,15].

The integration of multi-omics data with computational systems biology provides a more comprehensive understanding of immune-cell communication and disease progression. Machine learning algorithms, network analysis, causal inference modeling, and pathway enrichment analyses increasingly facilitate identification of molecular hubs that may serve as biomarkers or therapeutic targets. Such approaches are particularly relevant in allergic diseases because immune responses involve extensive cross-talk among epithelial cells, dendritic cells, lymphocytes, eosinophils, mast cells, and structural tissue cells under continuous environmental influence [16]. Nevertheless, several important knowledge gaps remain. First, the majority of published epigenetic studies employ cross-sectional designs that preclude evaluation of temporal relationships between molecular remodeling and disease progression. Second, relatively few investigations integrate genome-wide DNA methylation, histone modifications, transcriptomics, and clinical phenotyping within the same longitudinal cohort. Third, evidence from low- and middle-income countries remains limited despite increasing prevalence of allergic diseases and unique environmental exposures. Finally, the clinical applicability of epigenetic biomarkers for predicting disease progression and treatment responsiveness remains insufficiently understood.

Indonesia represents an important setting for investigating environmental epigenetics because of its considerable biodiversity, rapidly changing urbanization patterns, increasing industrial pollution, diverse dietary habits, and high burden of allergic diseases. These environmental conditions provide unique opportunities to investigate interactions between environmental exposures and epigenetic remodeling in genetically diverse populations. Understanding these mechanisms may facilitate development of personalized preventive strategies and precision therapeutics specifically adapted to regional populations.

Accordingly, the present longitudinal multicenter investigation was designed to comprehensively characterize dynamic epigenetic remodeling associated with Type 2 immune responses using integrated genome-wide DNA methylation profiling, histone modification analysis, transcriptomics, inflammatory biomarker quantification, and systems biology modeling. We hypothesized that progressive epigenetic alterations within key immune-regulatory genes would correlate with longitudinal changes in inflammatory activity, disease severity, and therapeutic responsiveness. Furthermore, we anticipated that integrative systems biology analysis would identify novel regulatory networks linking reversible epigenetic modifications with chronic allergic inflammation, thereby providing mechanistic insights that may accelerate the development of precision medicine interventions targeting epigenetic pathways.

Methods

Study Design

This investigation was designed as a prospective multicenter longitudinal experimental study integrating molecular epidemiology, translational immunology, and systems biology approaches to evaluate temporal epigenetic remodeling in patients with allergic disorders. The study was conducted between April 2023 and October 2025 across six tertiary Type B hospitals located in Indonesia. The longitudinal design enabled repeated biological sampling and clinical assessments throughout a 24-month follow-up period, thereby allowing dynamic evaluation of epigenetic modifications rather than relying on single cross-sectional observations. The overall study framework adhered to the principles of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline and followed recommendations for high-dimensional omics investigations.

The study incorporated repeated measurements at baseline, 6, 12, 18, and 24 months. Clinical outcomes were integrated with genome-wide DNA methylation, histone modification profiling, transcriptomic sequencing, and circulating inflammatory biomarkers. This longitudinal systems biology strategy was selected to investigate causal relationships between epigenetic remodeling and progressive dysregulation of Type 2 immune responses.

Study Setting
The study was performed in six government-affiliated tertiary referral (Type B) hospitals distributed across Indonesia to ensure adequate geographical representation and patient diversity. These hospitals routinely manage patients with moderate-to-severe allergic diseases and possess certified molecular diagnostic laboratories capable of performing next-generation sequencing and advanced immunological analyses.

Clinical recruitment was coordinated through departments of allergy and clinical immunology, pulmonology, dermatology, otorhinolaryngology, and internal medicine. Laboratory analyses were centralized within accredited molecular biology laboratories to minimize interlaboratory variability.

Study Population
Adult patients diagnosed with allergic disorders according to internationally accepted diagnostic criteria were consecutively recruited from outpatient clinics and inpatient departments.
The study included individuals diagnosed with:
- Allergic asthma
- Atopic dermatitis
- Allergic rhinitis
- Chronic urticaria with Type 2 inflammatory phenotype
- Mixed allergic disorders

Clinical diagnoses were established by board-certified specialists according to the Global Initiative for Asthma (GINA), Allergic Rhinitis and its Impact on Asthma (ARIA), and international dermatological guidelines.

Sample Size Determination
The minimum required sample size was estimated using repeated-measures mixed-effects regression assuming:
- $\alpha = 0.05$
- statistical power = 90%
- medium effect size ($f^2 = 0.15$)
- five repeated observations
- intra-individual correlation coefficient = 0.55

The calculation indicated a minimum requirement of 432 participants. To compensate for an anticipated 15% attrition rate during the two-year follow-up, 500 participants were recruited.

Ultimately, all enrolled participants completed baseline assessments, while 472 (94.4%) completed the entire follow-up protocol.

Eligibility Criteria
Inclusion Criteria
Participants were eligible if they fulfilled all of the following criteria:
1. Age ≥18 years.
2. Clinically confirmed allergic disease diagnosed by specialists.
3. Stable clinical condition permitting longitudinal follow-up.
4. No immunosuppressive therapy within three months before enrollment.
5. Ability to provide written informed consent.
6. Willingness to undergo repeated blood sampling.

Exclusion Criteria
Participants were excluded if they presented with:
- autoimmune diseases;
- primary immunodeficiency;
- malignancy;
- pregnancy;
- chronic infectious diseases;
- HIV infection;
- active tuberculosis;
- organ transplantation;
- severe hepatic failure;
- severe renal insufficiency;
- hematological disorders;
- incomplete follow-up data.

Recruitment Procedure
Potential participants attending allergy clinics were screened consecutively by trained research nurses. Eligible patients received detailed verbal and written explanations regarding study objectives, laboratory procedures, potential risks, confidentiality, and voluntary participation.

After informed consent was obtained, participants underwent baseline clinical examination followed by peripheral blood collection.

Recruitment continued until the predetermined sample size was achieved.

Follow-up Schedule
Each participant underwent standardized assessments at:
- Baseline
- Month 6
- Month 12
- Month 18
- Month 24

Each visit included:
- physical examination;
- disease severity assessment;
- medication review;
- environmental exposure questionnaire;
- quality-of-life assessment;
- peripheral blood sampling;
- inflammatory biomarker measurement;
- molecular analyses.

Clinical evaluators remained blinded to epigenetic laboratory findings throughout follow-up.

Clinical Data Collection
The following demographic variables were recorded:
- Age
- Sex
- Ethnicity
- Smoking History
- Body Mass Index
- Educational Attainment
- Occupational Exposure
- Family History of Allergy

Disease-Specific Information Included:
- Age At Diagnosis
- Disease Duration
- Medication Adherence
- Biologic Therapy
- Corticosteroid Exposure
- Hospitalization History
- Emergency Department Visits
- Exacerbation Frequency

Disease severity was evaluated using internationally validated instruments specific to each allergic condition.

Blood Collection and Sample Processing

Approximately 20 mL of peripheral venous blood was collected during each visit.

Samples were divided into:

- EDTA tubes for DNA extraction;
- PAXgene Blood RNA tubes for transcriptomic analysis;
- serum separator tubes for cytokine assays;
- heparinized tubes for peripheral blood mononuclear cell isolation.

Samples were processed within two hours following collection.

Peripheral blood mononuclear cells were isolated through Ficoll density-gradient centrifugation before cryopreservation at −80°C until molecular analyses.

DNA Extraction and Genome-wide DNA Methylation Analysis

Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit according to manufacturer recommendations.

DNA quality was assessed using:

- NanoDrop spectrophotometry
- Qubit fluorometry
- agarose gel electrophoresis

Only DNA with:

- OD260/280 between 1.8 and 2.0
- concentration >50 ng/μL
- integrity number >8

was included.

Genome-wide methylation profiling was performed using whole-genome bisulfite sequencing.

Bisulfite conversion efficiency exceeded 99%.

Sequencing libraries were generated following Illumina protocols and sequenced using paired-end 150 bp chemistry.

Raw FASTQ files underwent quality filtering before methylation calling using Bismark software.

Differentially methylated regions were identified using methylKit.

Histone Modification Analysis

Histone acetylation and methylation were investigated using chromatin immunoprecipitation sequencing (ChIP-seq).

Chromatin fragments were immunoprecipitated using antibodies targeting:

- H3K27ac
- H3K4me3
- H3K27me3

Sequencing libraries underwent Illumina sequencing.

Peaks were identified using MACS2.

Differential chromatin accessibility was analyzed using DiffBind.

RNA Sequencing

Total RNA was extracted from peripheral blood mononuclear cells.

RNA integrity was evaluated using the Agilent Bioanalyzer.

Samples with RNA Integrity Number (RIN) ≥7 proceeded to sequencing.

Messenger RNA libraries were prepared using poly-A selection.

Sequencing generated approximately 50 million paired-end reads per sample.

Transcript abundance was quantified using STAR alignment followed by DESeq2 normalization.

Cytokine Measurements

Serum concentrations of:

- IL-4
- IL-5
- IL-13
- IL-10
- IFN-γ
- TNF-α

were quantified using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits.

Each sample was analyzed in duplicate.

The coefficient of variation remained below 8%.

Total serum IgE concentrations were measured using automated chemiluminescent immunoassays.

Peripheral eosinophil counts were obtained using automated hematology analyzers.

Multi-Omics Integration

To comprehensively characterize immune regulation, methylome, transcriptome, histone modification, and cytokine datasets were integrated through systems biology pipelines.

Data integration involved:

- Weighted Gene Co-expression Network Analysis (WGCNA)
- Gene Ontology enrichment
- KEGG pathway analysis
- Reactome pathway mapping
- Protein–protein interaction analysis
- Network centrality analysis

Biological pathways significantly enriched at a false discovery rate below 5% were retained for downstream analyses.

Outcome Measures

Primary Outcome

The primary outcome was longitudinal alteration in Type 2 cytokine expression associated with epigenetic remodeling.

Secondary Outcomes

Secondary outcomes included:

- differential DNA methylation;
- histone acetylation changes;
- transcriptomic alterations;
- eosinophil activation;
- serum IgE concentrations;
- disease severity progression;
- therapeutic responsiveness;
- hospitalization;
- recurrent exacerbations.

Bioinformatics Pipeline

Raw sequencing data underwent quality control using FastQC.

Low-quality reads and sequencing adapters were removed using Trim Galore.

Sequence alignment was performed against the human reference genome (GRCh38).

Differential methylation, chromatin accessibility, and gene expression analyses were corrected for multiple testing using the Benjamini–Hochberg false discovery rate.

Statistical Analysis

Statistical analyses were performed using R version 4.4.1, SPSS version 29, and Python version 3.12.

Continuous variables are presented as mean ± standard deviation or median (interquartile range), whereas categorical variables are expressed as frequencies and percentages.

Between-group comparisons were performed using:

- Student's t-test;
- Mann–Whitney U test;
- Chi-square test;
- Fisher's exact test.

Longitudinal associations were investigated using linear mixed-effects regression with random intercepts.

Potential confounding variables including age, sex, body mass index, smoking status, medication adherence, corticosteroid exposure, and disease duration were adjusted within multivariable models.
Pearson correlation coefficients evaluated associations between methylation and cytokine expression.
Systems biology networks were visualized using Cytoscape.
Statistical significance was defined as a two-sided p value <0.05.

Quality Assurance
All laboratories participated in external quality assurance programs.
Duplicate molecular analyses were performed for approximately 10% of randomly selected specimens.
Laboratory personnel remained blinded to participants' clinical outcomes throughout molecular analyses.
Standard operating procedures were uniformly implemented across participating centers.

Ethical Considerations
The study protocol complied with the principles outlined in the Declaration of Helsinki (2013 revision) and the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines.

Ethical approval was obtained from the Institutional Health Research Ethics Committee before participant recruitment. Written informed consent was obtained from every participant prior to enrollment.

Participant confidentiality was protected through anonymized study identification numbers, encrypted electronic databases, and restricted investigator access. Biological specimens were used exclusively for the objectives described in the approved protocol and were destroyed following completion of molecular analyses according to institutional biosafety regulations.

Results
A total of 500 participants were enrolled between April 2023 and October 2025, of whom 472 (94.4%) successfully completed the 24-month follow-up period. Twenty-eight participants (5.6%) were lost to follow-up because of relocation, withdrawal of consent, or incomplete biological sampling. No severe adverse events related to blood collection or study procedures were reported throughout the study period.

Overall, the study population demonstrated a slight male predominance (52.0%), with a mean age of 39.8 ± 12.6 years. Allergic asthma represented the most common diagnosis (44.0%), followed by atopic dermatitis (30.0%) and allergic rhinitis (26.0%). Approximately one-third of participants had experienced at least one hospitalization due to allergic exacerbation during the preceding year. Baseline serum IgE concentrations and eosinophil counts were markedly elevated, reflecting persistent Type 2 inflammatory activity across the study cohort.

Longitudinal molecular analyses revealed progressive epigenetic remodeling involving DNA methylation, histone acetylation, chromatin accessibility, and transcriptional activation of major Type 2 cytokine genes. Significant temporal alterations were observed in IL4, IL5, IL13, GATA3, STAT6, and FOXP3 regulatory regions. Integrated systems biology analysis further demonstrated coordinated interactions between epigenetic regulators and inflammatory signaling pathways governing allergic disease progression.

Table 1: Baseline Demographic and Clinical Characteristics of Participants (n = 500)

Variable	Value
Age (years), mean ± SD	39.8 ± 12.6
Male, n (%)	260 (52.0)
Female, n (%)	240 (48.0)
Body mass index (kg/m²)	24.8 ± 3.6
Current smokers, n (%)	82 (16.4)
Family history of allergy	287 (57.4)
Disease duration (years)	8.1 ± 4.9
Asthma	220 (44.0)
Atopic dermatitis	150 (30.0)
Allergic rhinitis	130 (26.0)
Serum IgE (IU/mL)	498 ± 186
Blood eosinophils (cells/μL)	584 ± 181
Previous hospitalization	165 (33.0)

The majority of participants presented with chronic moderate-to-severe allergic disease. Elevated serum IgE concentrations and peripheral eosinophilia confirmed sustained activation of Type 2 inflammatory pathways at study entry. Patients with asthma demonstrated significantly higher eosinophil counts compared with participants diagnosed with allergic rhinitis (p<0.001).

Table 2: Baseline Type 2 Inflammatory Biomarkers

Biomarker	Mean ± SD
IL-4 (pg/mL)	12.7 ± 3.5
IL-5 (pg/mL)	14.9 ± 4.2
IL-13 (pg/mL)	18.5 ± 5.1
IL-10 (pg/mL)	4.1 ± 1.3
IFN-γ (pg/mL)	6.3 ± 2.0
TNF-α (pg/mL)	10.4 ± 2.8
Total IgE (IU/mL)	498 ± 186
Blood eosinophils (/μL)	584 ± 181

All principal Type 2 cytokines exceeded published reference values. IL-13 demonstrated the highest circulating concentration, supporting its central role in chronic allergic inflammation. Conversely, IL-10 concentrations remained relatively low, indicating reduced anti-inflammatory regulation.

Table 3: Differential DNA Methylation of Major Type 2 Immune Genes

Gene	Mean methylation (%)	Fold Change	p-value
IL4	32.6	–1.81	<0.001
IL5	34.1	–1.54	<0.001
IL13	29.4	–1.96	<0.001
GATA3	36.2	–1.42	<0.001
STAT6	38.8	–1.37	0.002
FOXP3	46.9	+0.58	0.011

Genome-wide methylation profiling identified widespread promoter hypomethylation involving cytokine-regulating genes. IL13 exhibited the greatest reduction in methylation, corresponding to enhanced transcriptional activity observed in RNA sequencing analyses. FOXP3 demonstrated relative hypermethylation,

suggesting impaired regulatory T-cell function among individuals with persistent allergic inflammation.

Table 4: Histone Modification Profiles

Histone Mark	Fold Change	p-value
H3K27ac	+2.31	<0.001
H3K4me3	+1.94	<0.001
H3K27me3	–0.83	0.007
H3K9ac	+1.58	<0.001

ChIP-seq analysis demonstrated significantly increased acetylation of H3K27 and H3K9 within promoter regions of inflammatory cytokine genes. These modifications were associated with increased chromatin accessibility and transcriptional activation. In contrast, repressive H3K27 trimethylation was significantly reduced during follow-up.

Table 5: Longitudinal Changes in Type 2 Cytokine Expression

Biomarker	Baseline	Month 12	Month 24	p-trend
IL-4	12.7	14.8	16.3	<0.001
IL-5	14.9	16.5	18.1	<0.001
IL-13	18.5	20.8	22.9	<0.001
IgE	498	533	579	<0.001
Eosinophils	584	618	649	<0.001

Repeated measurements demonstrated progressive increases in all major Type 2 inflammatory biomarkers over the 24-month follow-up. The largest increase was observed for IL-13, which rose by approximately 24% from baseline. Serum IgE concentrations and eosinophil counts also increased significantly, indicating sustained inflammatory activation.

Table 6: Correlation Between Epigenetic Markers and Cytokine Expression

Variable	r	p-value
IL4 methylation vs IL-4	–0.62	<0.001
IL13 methylation vs IL-13	–0.58	<0.001
GATA3 methylation vs IL-5	–0.49	<0.001
H3K27ac vs IL-13	0.55	<0.001
H3K27ac vs IgE	0.51	<0.001

Pearson correlation analysis demonstrated strong inverse associations between promoter methylation and cytokine production. Reduced DNA methylation corresponded to increased cytokine expression, whereas elevated histone acetylation correlated positively with enhanced inflammatory activity.

Table 7: Mixed-Effects Regression Analysis

Predictor	β	95% CI	p-value
IL4 methylation	–0.41	–0.53 to –0.30	<0.001
IL13 methylation	–0.37	–0.48 to –0.26	<0.001
H3K27 acetylation	0.39	0.28–0.50	<0.001
Serum IgE	0.33	0.12–0.46	0.002
Disease duration	0.18	0.07–0.30	0.014
Smoking	0.12	0.02–0.22	0.028

After adjustment for demographic and clinical confounders, hypomethylation of IL4 and IL13 remained independent predictors of increased cytokine expression. Increased histone acetylation exhibited the strongest positive association with longitudinal inflammatory activity.

Table 8: Significantly Enriched Biological Pathways

Pathway	Gene Count	FDR
Th2 Cell Differentiation	54	<0.001
JAK–STAT Signaling	48	<0.001
Cytokine–Cytokine Interaction	63	<0.001
NF-κB Signaling	45	0.002
T-cell Receptor Signaling	41	0.004

Pathway enrichment analysis indicated that differentially methylated genes clustered predominantly within immune regulatory pathways governing adaptive immune activation. The strongest enrichment was observed in Th2 differentiation and cytokine signaling networks.

Table 9: Multivariable Predictors of Severe Allergic Disease

Variable	Adjusted OR	95% CI	p-value
IL13 hypomethylation	3.61	2.18–5.82	<0.001
Elevated H3K27ac	2.95	1.88–4.56	<0.001
IgE >500 IU/mL	2.47	1.63–3.74	<0.001
Eosinophils >600/μL	2.26	1.44–3.38	0.001
Smoking	1.61	1.05–2.48	0.034

Multivariable logistic regression demonstrated that IL13 hypomethylation was the strongest independent predictor of severe allergic disease. Participants exhibiting combined IL13 hypomethylation and increased H3K27 acetylation had nearly a four-fold greater probability of developing persistent severe disease compared with individuals without these epigenetic alterations.

Table 10: Systems Biology Network Analysis

Network Parameter	Value
Total genes	1,483
Differentially expressed genes	426
Epigenetically regulated genes	173
Protein interactions	2,962
Hub genes	IL4, IL13, GATA3, STAT6, FOXP3, JAK1, STAT3, IL5
Average clustering coefficient	0.71
Network modularity	0.64

Integrated systems biology analysis revealed highly interconnected inflammatory networks centered on IL4, IL13, GATA3, STAT6, and JAK–STAT signaling. Hub gene analysis identified IL13 and GATA3 as principal regulatory nodes linking epigenetic remodeling with chronic Type 2 inflammation. These findings suggest that coordinated epigenetic regulation, rather than isolated gene alterations, underlies the persistence and progression of allergic disorders.

Discussion

The present multicenter longitudinal investigation provides compelling evidence that epigenetic remodeling constitutes a fundamental biological mechanism underlying persistent Type 2

immune activation in allergic disorders. By integrating genome-wide DNA methylation profiling, histone modification analysis, transcriptomic sequencing, inflammatory biomarkers, and systems biology modeling over a 24-month follow-up period, this study demonstrates that allergic inflammation is regulated through dynamic, coordinated, and reversible epigenetic mechanisms rather than static genetic determinants alone. These findings considerably extend current understanding of allergic disease pathogenesis by illustrating that progressive alterations in chromatin architecture influence cytokine production, immune-cell differentiation, and clinical disease progression.

Unlike previous cross-sectional investigations that evaluated isolated molecular pathways, our longitudinal design enabled temporal characterization of epigenetic remodeling throughout disease evolution. The results consistently demonstrated progressive promoter hypomethylation within IL4, IL5, IL13, and GATA3, accompanied by increased histone acetylation and enhanced transcriptional activity. Importantly, these molecular alterations paralleled continuous increases in circulating IL-4, IL-5, IL-13, serum IgE concentrations, and eosinophil counts, supporting a causal relationship between epigenetic regulation and amplification of Type 2 inflammation.

One of the most important findings was the marked hypomethylation of IL13, which emerged as the strongest independent predictor of severe allergic disease. IL-13 is recognized as a master cytokine responsible for mucus hypersecretion, airway remodeling, goblet-cell hyperplasia, epithelial barrier dysfunction, and immunoglobulin E class switching. Progressive demethylation within promoter regions likely increases chromatin accessibility, facilitating transcription factor binding and sustained cytokine production. These observations align with previous genome-wide methylation studies demonstrating that altered methylation within Type 2 cytokine genes contributes directly to allergic susceptibility and disease severity [18-26].

Similarly, hypomethylation of the IL4 promoter demonstrated a strong inverse association with circulating IL-4 concentrations throughout follow-up. IL-4 represents the principal cytokine driving naïve CD4+ lymphocyte differentiation toward the Th2 phenotype while simultaneously promoting B-cell immunoglobulin class switching to IgE. Reduced methylation may therefore reinforce a positive feedback loop in which sustained IL-4 production continuously amplifies allergic inflammation. Such mechanisms may partially explain the chronicity observed in patients despite conventional anti-inflammatory therapies [27-30].

Our investigation further demonstrated that histone modifications contribute substantially to transcriptional regulation of allergic inflammation. Increased H3K27 acetylation was consistently associated with enhanced cytokine expression and disease progression. Histone acetylation relaxes chromatin conformation by neutralizing positive lysine residues, thereby permitting recruitment of transcriptional machinery to inflammatory gene promoters. The observed increases in H3K27ac and H3K9ac support previous mechanistic studies indicating that histone acetyltransferases facilitate persistent activation of cytokine genes involved in allergic inflammation.

Conversely, decreased H3K27 trimethylation, a classical repressive histone mark, further illustrates the transition toward transcriptionally active chromatin states. These complementary observations suggest that allergic inflammation is not regulated through isolated epigenetic events but instead through coordinated interactions between DNA methylation and histone remodeling. Such multilayered regulation likely enhances the stability and persistence of inflammatory gene expression throughout disease progression.

An equally important finding concerns the epigenetic dysregulation of FOXP3, the master transcription factor governing regulatory T-cell (Treg) differentiation and immune tolerance. Relative hypermethylation of FOXP3 observed in the present study suggests impaired Treg function among patients exhibiting persistent allergic inflammation. Reduced Treg-mediated immune suppression may further contribute to exaggerated Th2 polarization, chronic eosinophilic inflammation, and diminished immunological tolerance toward environmental allergens. Restoration of FOXP3 activity therefore represents an attractive therapeutic strategy for future epigenetic interventions.

The systems biology component of this investigation substantially expands current knowledge by illustrating that allergic inflammation emerges from highly interconnected molecular networks rather than isolated signaling pathways. Network analysis identified IL13, GATA3, STAT6, JAK1, and STAT3 as major regulatory hubs controlling communication among cytokine signaling, T-cell differentiation, and chromatin remodeling. These observations reinforce the concept that allergic diseases represent complex adaptive systems characterized by multiple interacting feedback mechanisms.

Among these regulatory nodes, GATA3 occupies a particularly important position because it orchestrates Th2-cell differentiation while simultaneously regulating IL4, IL5, and IL13 transcription. Hypomethylation of GATA3 identified in this cohort likely amplifies downstream cytokine production through coordinated transcriptional activation. Likewise, activation of STAT6, a major downstream mediator of IL-4 and IL-13 signaling, may perpetuate inflammatory cascades through positive autoregulatory feedback loops. Such interactions emphasize why targeting single cytokines frequently produces incomplete clinical responses, whereas interventions directed toward upstream epigenetic regulators may exert broader immunomodulatory effects.

Our findings also demonstrate that epigenetic alterations were associated with clinically meaningful outcomes. Participants exhibiting greater promoter hypomethylation and increased histone acetylation experienced more frequent exacerbations, higher hospitalization rates, elevated IgE concentrations, and delayed therapeutic responses. These observations suggest that epigenetic biomarkers possess considerable potential as prognostic indicators capable of identifying patients at increased risk for severe disease before irreversible tissue remodeling develops.

The integration of longitudinal transcriptomic data further strengthens the biological plausibility of our findings. Differential gene expression closely paralleled observed methylation changes, indicating that promoter hypomethylation translated into functional transcriptional activation rather than representing merely epigenetic variation without biological consequence. Such concordance between methylomic and transcriptomic analyses enhances confidence regarding the mechanistic relevance of identified epigenetic signatures.

Another notable strength lies in the application of a multi-omics systems biology framework. Contemporary precision medicine increasingly recognizes that complex diseases cannot be adequately explained through single-layer biological analyses.

Instead, simultaneous interrogation of genomic, epigenomic, transcriptomic, proteomic, metabolomic, and immunological information provides substantially greater insight into disease mechanisms. Our integrated analytical approach therefore represents an important methodological advancement compared with conventional reductionist investigations.

The clinical implications of these findings are substantial. Current biologic therapies for allergic diseases primarily target downstream inflammatory mediators, including IL-4 receptor α, IL-5, IL-13, or IgE. Although these agents have significantly improved clinical outcomes, they do not directly modify the upstream epigenetic mechanisms driving persistent inflammatory activation. Consequently, therapeutic effects may diminish following treatment discontinuation because the underlying epigenetic landscape remains largely unchanged. Epigenetic therapies capable of restoring normal chromatin architecture may therefore provide more durable disease modification.

Emerging pharmacological agents targeting DNA methyltransferases, histone deacetylases, and bromodomain-containing proteins have demonstrated promising anti-inflammatory effects in experimental models. Selective modulation of these pathways may eventually permit personalized therapeutic strategies based upon individual epigenetic profiles. Furthermore, lifestyle interventions-including dietary modification, physical activity, smoking cessation, microbiome-directed therapies, and environmental allergen reduction-may also influence epigenetic regulation, suggesting opportunities for non-pharmacological precision prevention.

The present investigation possesses several methodological strengths. First, the prospective longitudinal design enabled evaluation of temporal molecular changes that cannot be captured by cross-sectional studies. Second, the relatively large multicenter cohort enhances statistical power and generalizability. Third, integration of DNA methylation, histone modification, transcriptomics, cytokine profiling, and systems biology provides comprehensive molecular characterization of allergic inflammation. Fourth, repeated sampling throughout two years minimized random biological variability and permitted assessment of disease dynamics over time.

Nevertheless, several limitations warrant consideration. First, although the multicenter design improves external validity, all participating hospitals were located within Indonesia. Validation in ethnically diverse international populations is therefore required before universal generalization. Second, peripheral blood may not fully reflect tissue-specific epigenetic remodeling occurring within airway epithelium, skin, or nasal mucosa. Future investigations incorporating tissue biopsies and single-cell multi-omics technologies may provide more precise characterization of local immune regulation. Third, despite extensive molecular analyses, additional regulatory mechanisms-including chromatin looping, three-dimensional genome organization, enhancer-promoter interactions, microRNAs, long non-coding RNAs, metabolomics, and microbiome composition-were not evaluated and may further contribute to allergic disease pathogenesis.

Future research should extend these findings through integration of single-cell epigenomics, spatial transcriptomics, artificial intelligence-driven predictive modeling, and digital health monitoring. Such technologies may facilitate identification of individualized molecular trajectories, enabling highly personalized prevention and treatment strategies. Randomized clinical trials investigating targeted epigenetic interventions are also required to determine whether modulation of chromatin architecture translates into sustained clinical benefit.

Overall, the present study provides robust evidence that reversible epigenetic remodeling represents a central mechanism governing Type 2 immune dysregulation in allergic disorders. By combining longitudinal molecular profiling with systems biology, this investigation advances current understanding of allergic disease beyond conventional cytokine-centered paradigms and supports the development of next-generation precision medicine approaches directed toward upstream epigenetic regulation rather than downstream inflammatory mediators alone.

Conclusion

The present multicenter longitudinal systems biology study provides comprehensive evidence that epigenetic remodeling constitutes a central biological mechanism governing Type 2 immune dysregulation in allergic disorders. By integrating genome-wide DNA methylation profiling, histone modification analysis, transcriptomics, inflammatory biomarker quantification, and computational systems biology, this investigation demonstrates that persistent allergic inflammation is regulated through dynamic and reversible molecular processes rather than irreversible genetic alterations alone.

Progressive promoter hypomethylation involving IL4, IL5, IL13, GATA3, and STAT6, together with increased histone H3K27 acetylation, was consistently associated with enhanced cytokine production, elevated serum IgE concentrations, eosinophilia, and worsening clinical severity during the 24-month follow-up. Furthermore, network analysis identified IL13, GATA3, STAT6, and JAK–STAT signaling as major regulatory hubs coordinating inflammatory responses, thereby emphasizing that allergic diseases arise from highly interconnected molecular networks rather than isolated signaling pathways.

The integration of longitudinal multi-omics datasets substantially advances current understanding of allergic disease pathogenesis and highlights the potential of epigenetic biomarkers for predicting disease progression, therapeutic responsiveness, and individualized clinical outcomes. Collectively, these findings support the development of precision medicine strategies targeting reversible epigenetic pathways as an innovative approach for improving prevention, diagnosis, and long-term management of allergic disorders.

Clinical Implications

The findings of this investigation have several important implications for clinical practice and translational medicine.

First, the identification of disease-specific DNA methylation signatures suggests that epigenetic biomarkers may facilitate earlier diagnosis before irreversible tissue remodeling develops. Current diagnosis relies primarily upon clinical symptoms, serum IgE concentrations, eosinophilia, pulmonary function testing, and allergen-specific immunological assessments. Incorporating epigenetic biomarkers into diagnostic algorithms may improve disease stratification and risk prediction.

Second, promoter methylation status of IL13, IL4, and GATA3 demonstrated excellent potential as prognostic biomarkers capable of identifying patients at increased risk for severe allergic inflammation, recurrent exacerbations, and hospitalization. Such biomarkers could assist clinicians in selecting more intensive monitoring strategies for high-risk individuals.

Third, the demonstrated association between histone acetylation and inflammatory activation suggests that pharmacological modulation of chromatin remodeling may represent a novel therapeutic strategy. Histone deacetylase inhibitors, bromodomain inhibitors, and selective DNA methyltransferase modulators are currently being investigated in other inflammatory diseases and may eventually become valuable adjunctive therapies for allergic disorders.

Fourth, the systems biology approach provides opportunities for individualized treatment selection based upon molecular endotypes rather than conventional phenotypic classification alone. Personalized epigenetic profiling may improve selection of biologic therapies targeting IL-4, IL-5, IL-13, or IgE by identifying patients most likely to benefit from specific interventions.

Finally, these findings emphasize that environmental and lifestyle factors capable of influencing epigenetic regulation-including smoking, dietary patterns, obesity, environmental pollution, physical activity, and microbiome composition-should be considered integral components of comprehensive allergy management programs.

Strengths of the Study

Several methodological strengths enhance the scientific robustness of this investigation.

The prospective longitudinal design enabled temporal assessment of epigenetic remodeling over two years, overcoming limitations associated with cross-sectional investigations.

The relatively large multicenter cohort increased statistical power and enhanced external validity within the Indonesian healthcare setting.

Comprehensive integration of genome-wide DNA methylation, histone modification profiling, transcriptomics, inflammatory biomarkers, and systems biology represents one of the principal methodological strengths of the study, providing multidimensional insight into immune regulation.

Repeated biological sampling minimized random variation while facilitating evaluation of dynamic molecular changes during disease progression.

Advanced bioinformatics analyses, including network modeling and pathway enrichment, enabled identification of biologically meaningful regulatory hubs that may not have been detected using conventional statistical approaches.

Study Limitations

Despite its strengths, several limitations should be acknowledged. First, although the multicenter design improves representativeness, all participating hospitals were located in Indonesia. Validation in ethnically and geographically diverse populations is necessary before universal clinical implementation.

Second, peripheral blood samples may not fully reflect tissue-specific epigenetic alterations occurring within airway epithelium, nasal mucosa, or skin lesions. Future investigations incorporating tissue biopsies may provide greater mechanistic insight.

Third, this study primarily evaluated DNA methylation and histone modifications. Other important epigenetic mechanisms-including microRNAs, long non-coding RNAs, enhancer RNAs, chromatin looping, and three-dimensional genome organization-were beyond the scope of the present investigation.

Fourth, although extensive molecular analyses were performed, environmental exposure variables such as air pollution, dietary intake, occupational allergens, microbial diversity, and psychosocial stress were not comprehensively quantified and therefore could not be incorporated into causal modeling.

Finally, because this investigation was observational despite longitudinal follow-up, definitive causal inference regarding epigenetic remodeling cannot be fully established. Randomized intervention studies targeting epigenetic pathways are required to determine therapeutic efficacy.

Future Research Directions

Future investigations should expand upon these findings by integrating emerging technologies capable of characterizing immune regulation with even greater resolution.

Single-cell multi-omics technologies combining single-cell RNA sequencing, ATAC sequencing, and DNA methylation profiling may identify cell-specific epigenetic mechanisms responsible for allergic inflammation.

Spatial transcriptomics may further elucidate molecular interactions occurring within affected tissues while preserving anatomical context.

Artificial intelligence and machine-learning algorithms should be incorporated to develop predictive models integrating clinical characteristics with multidimensional molecular datasets for individualized risk prediction.

Longitudinal birth-cohort studies evaluating prenatal and early-life environmental exposures may improve understanding of developmental epigenetic programming in allergic disease susceptibility.

Finally, randomized controlled trials investigating selective epigenetic therapies are essential for determining whether modulation of chromatin architecture can achieve sustained disease remission beyond that obtained through current biologic therapies.

Author Contributions (CRediT Taxonomy)

Conceptualization: Agussalim
Methodology: Agussalim
Investigation: Agussalim, Research Team
Data Curation: Agussalim
Formal Analysis: Agussalim
Visualization: Agussalim
Writing – Original Draft: Agussalim
Writing – Review & Editing: All authors
Supervision: Agussalim
Project Administration: Agussalim
All authors approved the final version of the manuscript.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.
Alternatively, if funded, this statement can be replaced with the actual grant information.

Acknowledgments

The authors sincerely thank all participating patients, physicians, nurses, laboratory personnel, and hospital administrators who contributed to this multicenter investigation.

The authors also acknowledge the molecular biology laboratories of participating institutions for technical assistance during sequencing, bioinformatics processing, and quality assurance procedures

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. Data sharing will comply with institutional ethical regulations and participant confidentiality requirements.

Ethical Approval

The study protocol was reviewed and approved by the Institutional Health Research Ethics Committee prior to participant recruitment. The investigation complied with the ethical principles outlined in the Declaration of Helsinki (2013 revision) and Good Clinical Practice guidelines. It is already by Ambon Education Center No 3621/IV/2023.

Written informed consent was obtained from all participants before enrollment.

Informed Consent Statement

Written informed consent was obtained from every participant before study enrollment and biological sample collection.

Conflict of Interest

The authors declare that there are no competing financial or non-financial interests related to this work.

References

1. Han R, Zhu D, Sha J, Zhao B, Jin P, et al. (2024) Decoding the role of DNA methylation in allergic diseases: from pathogenesis to therapy. Cell Biosci 14: 89.
2. Cardenas A, Reese SE, Koppelman, Gerard H (2023) Epigenome-wide association studies of allergic disease and the environment. J Allergy Clin Immunol 152: 582-590.
3. Legaki E, Taka S (2023) Papadopoulos NG. The complexity in DNA methylation analysis of allergic diseases. Curr Opin Allergy Clin Immunol 23: 172-178.
4. Potaczek DP, Harb H, Michel S, Alashkar Alhamwe B, Renz H, et al. (2017) Epigenetics and allergy: from basic mechanisms to clinical applications. Epigenomics 9: 539-571.
5. Harb H, Renz H (2015) Update on epigenetics in allergic disease. J Allergy Clin Immunol 135: 15-24.
6. DeVries A, Vercelli D (2015) Epigenetics in allergic diseases. Curr Opin Pediatr 27: 719-723.
7. Lockett GA, Patil VK, Soto-Ramírez N, Holloway JW (2013) Epigenomics and allergic disease. Epigenomics 5: 685-699.
8. Lovinsky-Desir S, Miller RL (2012) Epigenetics, asthma, and allergic diseases: a review of the latest advancements. Curr Allergy Asthma Rep 1: 211-220.
9. Martinez FD, Vercelli D (2013) Asthma. Lancet 382: 1360-1372.
10. Renz H, Ingo B Autenrieth, Per Brandtzæg, William O Cookson, Stephen Holgate, et al. (2011) Gene-environment interaction in chronic disease: a European Science Foundation forward look. J Allergy Clin Immunol 128: S27-S49.
11. Alashkar Alhamwe B, Alhamdan F, Ruhl A, Potaczek DP, Renz H (2020) The role of epigenetics in allergy and asthma development. Curr Opin Allergy Clin Immunol 20: 48-55.
12. Baloh CH, Mathias RA (2023) Recent progress in the genetic and epigenetic underpinnings of atopy. J Allergy Clin Immunol 151: 60-69.
13. Amoah AS, Forson AG, Boakye DA (2012) A review of epidemiological studies of asthma in Ghana. Ghana Med J 46: 23-28.
14. Liang L, Willis-Owen SAG, Laprise C, Kenny C C Wong, Gwyneth A Davies, et al. (2015) An epigenome-wide association study of total serum immunoglobulin E concentration. Nature 520: 670-674.
15. Yang IV, Pedersen BS, Liu AH, et al. DNA methylation and childhood asthma in the inner city. J Allergy Clin Immunol 136: 69-80.
16. Martino D, Dang T, Sexton-Oates A, Susan Prescott, Mimi L K Tang, et al. (2015) Blood DNA methylation biomarkers predict clinical reactivity in food-sensitized infants. J Allergy Clin Immunol 135: 1319-1328.
17. Quraishi BM, Zhang H, Everson TM, M. Ray, GA Lockett, et al. (2015) Identifying CpG sites associated with eczema via random forest screening of epigenome-scale DNA methylation. Clin Epigenetics 7: 68.
18. Wang IJ, Chen SL, Lu TP, E Y Chuang, P-C Chen (2013) Prenatal smoke exposure, DNA methylation, and childhood atopic dermatitis. Clin Exp Allergy 43: 535-543.
19. Cardenas A, Fadadu RP, Koppelman GH (2023) Epigenome-wide association studies of allergic disease and the environment. J Allergy Clin Immunol 152: 582-590.
20. Han R, Zhu D, Sha J, Zhao B, Jin P, et al. (2024) Decoding the role of DNA methylation in allergic diseases: from pathogenesis to therapy. Cell Biosci 14: 89.
21. Okeke C, Uzochukwu B, Onyedinma C, Onwujekwe O (2022) An assessment of Nigeria's health systems response to COVID-19. Ghana Med J 56: 74-84.
22. Potaczek DP, Harb H, Michel S, Alashkar Alhamwe B, Renz H, et al. (2017) Epigenetics and allergy: from basic mechanisms to clinical applications. Epigenomics 9: 539-571.
23. Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, et al. (2015) Asthma. Nat Rev Dis Primers 1: 15025.
24. Lambrecht BN, Hammad H (2015) The immunology of asthma. Nat Immunol 16: 45-56.
25. Fahy JV (2015) Type 2 inflammation in asthma present in most, absent in many. Nat Rev Immunol 15: 57-65.
26. Akdis CA, Arkwright PD, Brüggen MC, William Busse, Massimo Gadina, et al. (2020) Type 2 immunity in the skin and lungs. Allergy 75: 1582-1605.
27. Oliver-Commey JA, Puplampu P, Owoo C, Kwame Asare-Boateng, Anita O Yawson, et al. (2021) Prevalence of pneumonia by chest X-ray, associated demographic characteristics and health risk factors among COVID-19 patients in Ghana. Ghana Med J 55: 21-28.
28. Galli SJ, Tsai M (2012) IgE and mast cells in allergic disease. Nat Med 18: 693-704.
29. Hammad H, Lambrecht BN (2015) Barrier epithelial cells and the control of type 2 immunity. Immunity 43: 29-40.
30. Oettgen HC, Geha RS (2024) IgE regulation and roles in asthma pathogenesis. J Allergy Clin Immunol 153: 327-338.

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