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Computational Analysis of Genetic Variants Impacting IgE Regulation and Asthma Susceptibility

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ABSTRACT

Asthma is a complex and variable condition influenced by genetic and environmental factors, with IL-13 and IgE playing critical roles in its development. IL-13 contributes to airway inflammation by promoting IgE production, which in turn triggers allergic responses and immune cell activation. While IL-13 and IL-4 share receptor components and functions, IL-13 has distinct effects that contribute to chronic inflammation and airway remodeling. Understanding the molecular interactions between IL-13 and IgE is essential for uncovering new therapeutic targets.

This study employs a bioinformatics-driven approach to explore the role of IL-13 and IgE in asthma. Using databases such as KEGG, GeneCards, and ImmPort, we analyzed key genes, pathways, and immune responses associated with asthma. Protein structure predictions from AlphaFold and stability analysis using DynaMut2 allowed us to examine the impact of mutations in IL-13 on its function.

By integrating genomic, proteomic, and structural data, this research highlights critical molecular mechanisms underlying asthma pathogenesis. The findings contribute to a deeper understanding of how IL-13 influences IgE-mediated immune responses, offering new perspectives on disease progression and potential biomarkers. While further experimental validation is needed, these insights lay the foundation for future studies aimed at developing more targeted and personalized asthma treatments.

Keywords: Asthma, Immunoglobulin E, IL-13, Bioinformatics tools, Protein structure prediction.

1. INTRODUCTION

The chronic condition known as asthma causes repeated, varied symptoms like coughing, wheezing, dyspnea, facilitated by variable airflow restriction, airway inflammation and the airways hyper-responsiveness[1]. According to the World Health Organization (WHO), asthma affects over 340 million people worldwide and is the most prevalent persistent health condition among children[2]. When an inhaled allergen is encountered by antigen-presenting cells (APCs) of lining of respiratory passages, an allergic process is initiated. Following the recognition of these antigens and subsequent activation of the APCs, naive T cells undergo differentiation, activating certain T helper cells. These activated Th2 cells then play a role in promoting the production of IgE antibodies by B cells[3]. IgE antibodies attach to specific receptors on the surface of mast cells. When allergens crosslink these IgE molecules on mast cells, it triggers degranulation, resulting in the release of biologically active substances such as histamine and leukotrienes, which are responsible for the immediate allergic symptoms. Additionally, mast cells secrete chemotactic factors that facilitate the recruitment of inflammatory cells—most notably eosinophils. The proliferation and differentiation of eosinophils from their bone marrow progenitors are primarily driven by interleukin-5 (IL-5)[4].

Allergic asthma is a complex disease influenced by both genetic and environmental factors. It is characterized by bronchial hyperresponsiveness, the presence of IgE antibodies against inhaled allergens, and often an overall increase in total serum IgE levels[5]. For B cells to undergo class switching for IgE production, they require two crucial signals originating from activated T cells such as the presence of CD40 ligand on the T cell surface and the release of interleukin-4 (IL-4) or interleukin-13 (IL-13). Importantly, both IL-4 and IL-13 can individually stimulate the production of IgE antibodies[6]. Beyond their role in IgE production, IL-4 and IL-13 share several other functions, including the up-regulation of CD23 on B lymphocytes and monocytes, as well as the suppression of cytokine release in LPS-activated monocytes. They also influence the secretion of various proinflammatory and immunomodulatory cytokines such as interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-

8), interleukin-10 (IL-10), interleukin-12 (IL-12), tumor necrosis factor-alpha (TNF-α), and interferon-alpha (IFN-α), MIP-1α, and GM-CSF, while simultaneously upregulating IL-1Ra. Despite some functional similarities, IL-4 and IL-13 exhibit distinct characteristics. Although interleukin-4 (IL-4) functions as a growth factor for T cells, interleukin-13 (IL-13) lacks this property. Both cytokines, however, can activate Stat-6 in T cells of peripheral blood, which is essential for Th2 differentiation. Interestingly, IL-13 alone is insufficient to generate Th2 differentiation in human naive T cells. The overlapping functions of Interleukin-4 and Interleukin-13 can be attributed to their shared receptor components. The IL-4 receptor α-chain serves as a component for the receptor of IL-13 as well. Furthermore, two distinct binding subunits specific to Interleukin-13, known as IL-13Rα1 and IL-13Rα2, have been identified. Notably, IL-4 may also interact with these subunits. While T cells primarily express IL-13Rα1, its expression rapidly diminishes after activation, which could account for IL-13's persistent biological activity in T cells[7].

Recent studies on allergic asthma suggest that epithelial-derived cytokines, including IL-25, IL-33, and TSLP, along with CC chemokine family members such as MCP-1, MIP-1α, and MIP-1β, play a role in initiating allergic inflammation. They do this by recruiting inflammatory cells and activating dendritic cells. IL-33 stimulates mast cells to release their mediators. Additionally, both IL-25 and IL-33 activate IL-25 receptor-positive ($IL\text{-}25R^{+}$) lymphoid cells, leading to the production of IL-5 and IL-13. T cells differentiate into T follicular helper or Th2 cells, migrating to the lungs, where Th2 cells secrete cytokines such as IL-4, IL-5, and IL-13, which contribute to the recruitment and prolonged survival of basophils and eosinophils[8].

This study aims to utilize bioinformatics to explore how Interleukin-13 and IgE contribute to the pathogenic processes of asthma. By leveraging network analysis, and pathway enrichment tools, we will investigate the molecular interactions and regulatory mechanisms involving IL-13 and IgE. Specifically, we seek to identify key genes, signalling pathways, and immune cell responses influenced by IL-13 and IgE in asthma. Through integrative bioinformatics analysis, this study aims to provide novel insights into potential therapeutic targets and biomarkers for asthma management.

2. MATERIALS AND METHODS

2.1. ImmPort for Immunological Datasets

The Immunology Database and Analysis Portal (ImmPort)[9] (https://www.immport.org/)was utilized to retrieve immunological datasets, including IgE-related studies. ImmPort serves as a long-term, sustainable repository for immunology research data primarily supported by the National Institute of Allergy and Infectious Diseases (NIAID) and the Division of Allergy, Immunology, and Transplantation (DAIT). It integrates vast datasets from clinical trials and experiments, providing researchers with an immunology-focused ontology and data analytic tools. Registered and DAIT-approved researchers can access analytical tools and data during the embargo period. Data from researchers funded by NIAID/DAIT are made available to all registered users.

2.2. KEGG (Kyoto Encyclopedia of Genes and Genomes) for Pathway Mapping

The Kyoto Encyclopedia of Genes and Genomes (KEGG)[10] database (https://www.genome.jp/kegg/) was utilized to analyze molecular pathways associated with IL-13, IgE, and allergic asthma. KEGG provides a comprehensive resource for understanding biological pathways, gene functions, and protein interactions. The analysis focused on key pathways involving IL-13 and IgE, such as: JAK-STAT signaling pathway, Th2 cell differentiation and cytokine-cytokine receptor interactions.

2.3. GeneCards Database Analysis

The GeneCards database[11] (https://www.genecards.org/) was employed to retrieve comprehensive gene-related information associated with IL-13, IgE, and allergic asthma. GeneCards is an integrative database compiling genomic, transcriptomic, proteomic, and functional annotations from multiple sources.

To identify key genes involved in IL-13 and IgE signaling, relevant keywords such as "IL-13," "IgE," and "asthma" were searched in GeneCards. The retrieved data included gene function, expression profiles, interacting proteins, and associated pathways. Genes with high relevance scores were selected for further bioinformatics analysis, including pathway enrichment and network interaction studies. The obtained information was validated using KEGG databases to ensure functional interactions and biological significance.

2.4. UniProt Protein Analysis

The Universal Protein Resource[12] (UniProt) (https://www.uniprot.org/) was used to extract protein sequence and functional information for IL-13 and associated signaling molecules. The UniProtKB/Swiss-Prot section, known for its high-quality manually curated annotations, was prioritized.

For protein sequence and structural analysis, the following databases were used:

- UniProt: The leading resource for high-quality, freely accessible protein sequence and functional data.
- AlphaFold: AI-based protein structure prediction tool.
- RCSB PDB (Protein Data Bank): Repository for experimentally determined protein structures.

2.5. AlphaFold and RCSB PDB for Protein Structure Analysis

AlphaFold[13] is an AI-driven system that predicts a protein's 3D structure from its amino acid sequence by "AlphaFold Protein Structure Database" (https://alphafold.ebi.ac.uk/) . The Protein Data Bank file format is a textual standard employed to detail the three-dimensional architecture of molecules housed in the Protein Data Bank. The format is structured to accommodate descriptions and annotations of protein and nucleic acid structures, specifying atomic positions and their interactions. In addition, the experimental context of the structural data is preserved within the file. These structural files were used in downstream analyses, including stability predictions and molecular interaction studies.

2.6. DynaMut2 Structural and Stability Analysis

DynaMut2[14] (http://biosig.unimelb.edu.au/dynamut2) was used to predict the effects of single nucleotide polymorphisms (SNPs) or mutations on IL-13-related protein stability and flexibility. This tool integrates normal mode analysis (NMA) and molecular dynamics-based predictions to assess the impact of mutations on protein dynamics and stability.

Protein structure files (PDB format) for IL-13 and its receptor were obtained from the Protein Data Bank (PDB) and uploaded to DynaMut2. Point mutations of interest were introduced, and the resulting changes in Gibbs free energy (ΔΔG) and molecular flexibility were analyzed. Structural visualizations and interaction networks were examined to determine the potential effects of mutations on protein function and stability.

These analyses provide insights into the molecular pathways involved in IgE-mediated asthma pathology and potential therapeutic targets.

3. RESULT:

3.1. ImmPort Shared Data [The Immunology Database and Analysis Portal (ImmPort)]

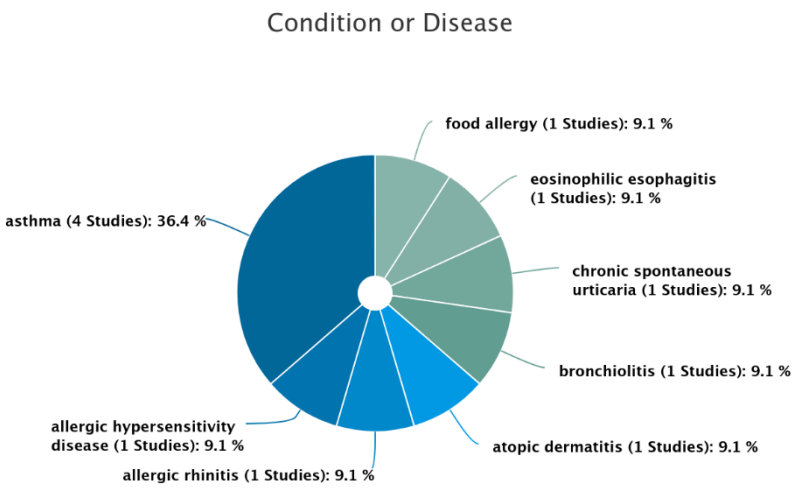
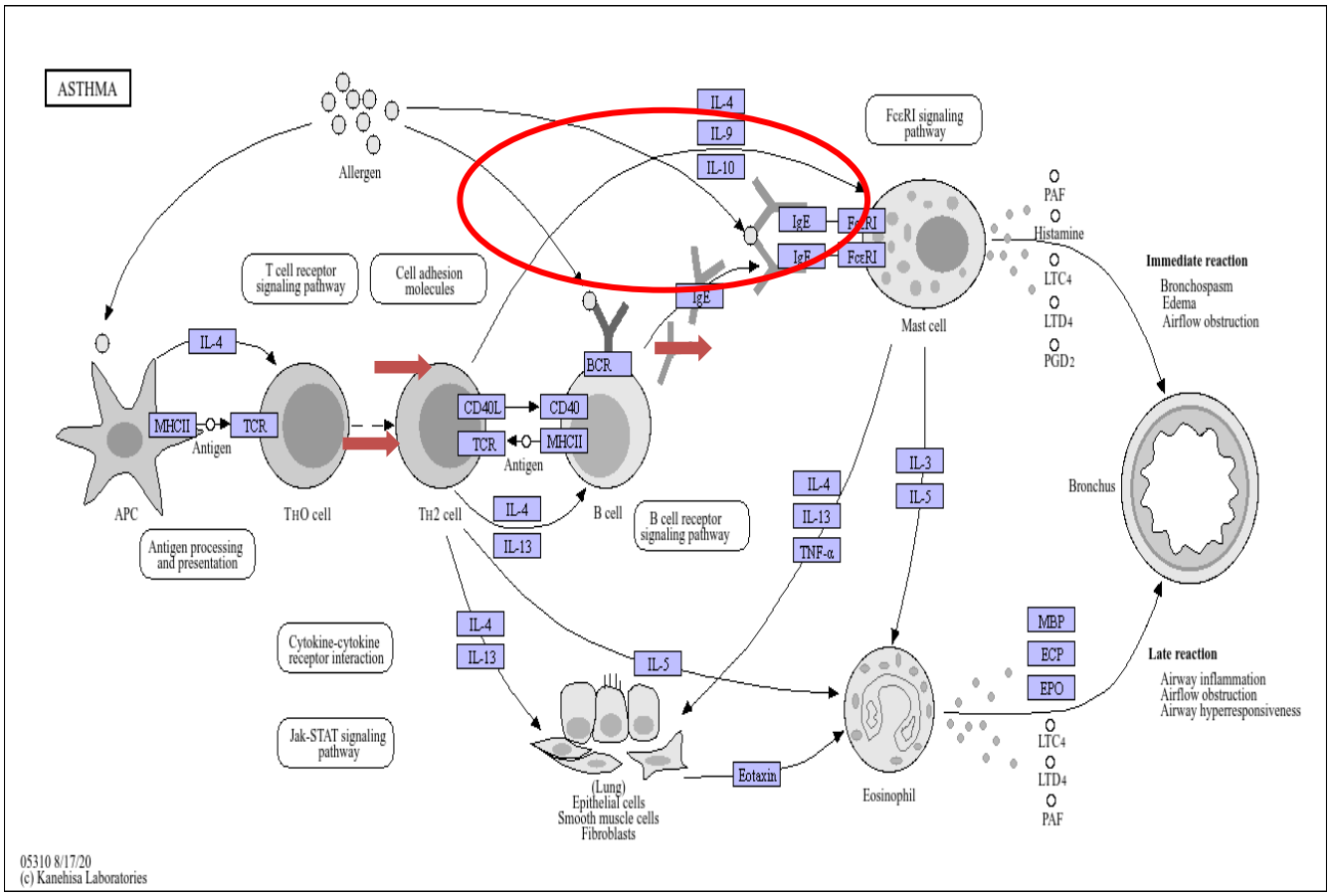


Figure 1: Distribution of studies investigating IgE levels across various allergic and immune-related conditions, as represented in ImmPort Search. (Source: ImmPort Search)

The pie chart from ImmPort Search represents the distribution of studies investigating IgE levels across various allergic and immune-related conditions. (Fig. 1) The analysis revealed that asthma accounts for 36.4% of the studies (4 studies), making it the most frequently investigated condition. This suggests a strong interest in IgE's role in asthma, likely due to its well-established connection to allergic responses and airway inflammation. Several other allergic and immune-mediated diseases were examined, each with one study (9.1%), including allergic rhinitis, atopic dermatitis, food allergy, bronchiolitis, chronic spontaneous urticaria, eosinophilic esophagitis, and allergic hypersensitivity disease. These conditions also involve IgE-related immune mechanisms but receive less research focus compared to asthma.

3.2. KEGG (Kyoto Encyclopedia of Genes and Genomes) for pathway mapping.



Source: KEGG PATHWAY Database (https://www.kegg.jp/kegg)

Figure 2: Schematic representation of the immunological mechanisms underlying asthma. The diagram illustrates the role of antigen-presenting cells (APCs) in antigen processing and presentation, leading to T-helper (Th0) cell activation and differentiation into Th2 cells. Key cytokines, including IL-4, IL-5, IL-9, IL-10, and IL-13, contribute to B-cell activation, IgE production, and mast cell sensitization via the FcεRI receptor.

The image strongly supports the correlation between elevated IgE levels and asthma pathogenesis (Fig.2). Increased IgE leads to mast cell degranulation, triggering both the immediate hypersensitivity response, characterized by bronchospasm, and a chronic inflammatory response driven by eosinophils. This underscores IgE as a critical biomarker and a key therapeutic target in asthma.

Table 1: List of human genes involved in asthma pathophysiology

Organism	Homo sapiens (human) [GN:hsa]
Gene	3108 HLA-DMA; major histocompatibility complex, class II, DM alpha [KO:K06752] 3109 HLA-DMB; major histocompatibility complex, class II, DM beta [KO:K06752] 3111 HLA-DOA; major histocompatibility complex, class II, DO alpha [KO:K06752] 3112 HLA-DOB; major histocompatibility complex, class II, DO beta [KO:K06752] 3113 HLA-DPA1; major histocompatibility complex, class II, DP alpha 1 [KO:K06752] 3115 HLA-DPB1; major histocompatibility complex, class II, DP beta 1 [KO:K06752] 3117 HLA-DQA1; major histocompatibility complex, class II, DQ alpha 1 [KO:K06752] 3118 HLA-DQA2; major histocompatibility complex, class II, DQ alpha 2 [KO:K06752] 3119 HLA-DQB1; major histocompatibility complex, class II, DQ beta 1 [KO:K06752] 3120 HLA-DQB2; major histocompatibility complex, class II, DQ beta 2 [KO:K06752] 3122 HLA-DRA; major histocompatibility complex, class II, DR alpha [KO:K06752] 3123 HLA-DRB1; major histocompatibility complex, class II, DR beta 1 [KO:K06752] 3125 HLA-DRB3; major histocompatibility complex, class II, DR beta 3 [KO:K06752] 3126 HLA-DRB4; major histocompatibility complex, class II, DR beta 4 [KO:K06752] 3127 HLA-DRB5; major histocompatibility complex, class II, DR beta 5 [KO:K06752] 3565 IL4; interleukin 4 [KO:K05430] 959 CD40LG; CD40 ligand [KO:K03161] 958 CD40; CD40 molecule [KO:K03160] 102723407 IGH; immunoglobulin heavy variable 4-38-2-like [KO:K06856] 2205 FCER1A; Fc epsilon receptor Ia [KO:K08089] 2206 MS4A2; membrane spanning 4-domains A2 [KO:K08090] 2207 FCER1G; Fc epsilon receptor Ig [KO:K07983] 3578 IL9; interleukin 9 [KO:K05432] 3586 IL10; interleukin 10 [KO:K05443] 3596 IL13; interleukin 13 [KO:K05435] 3567 IL5; interleukin 5 [KO:K05428] 6356 CCL11; C-C motif chemokine ligand 11 [KO:K16597] 7124 TNF; tumor necrosis factor [KO:K03156] 3562 IL3; interleukin 3 [KO:K04736] 5553 PRG2; proteoglycan 2, pro eosinophil major basic protein [KO:K10786] 6037 RNASE3; ribonuclease A family member 3 [KO:K10787] [EC:3.1.27.-] 8288 EPX; eosinophil peroxidase [KO:K10788] [EC:1.11.1.7]

Source: KEGG PATHWAY Database (https://www.kegg.jp/kegg)

The table 1 provides a list of human genes involved in asthma pathophysiology, particularly focusing on immune response elements such as major histocompatibility complex (MHC) class II molecules, cytokines (IL-4, IL-5, IL-9, IL-10, IL-13, TNF), chemokines (CCL11), receptors (CD40, FCER1A, FCER1G), and eosinophilic mediators (PRG2, RNASE3, EPX).

1. IgE Production and Regulation: IL-4 (Gene: 3565) and IL-13 (Gene: 3596) promote IgE class switching in B cells, driving allergic responses. CD40 (Gene: 958) and CD40LG (Gene: 959) are crucial in B cell activation and IgE production. IGH (Gene: 102723407) represents the immunoglobulin heavy chain, directly linked to IgE synthesis.
2. IgE-Mediated Mast Cell Activation: FCER1A (Gene: 2205), FCER1G (Gene: 2207), and MS4A2 (Gene: 2206) encode subunits of the high-affinity IgE receptor (FcεRI), which is essential for mast cell activation. Upon allergen binding, IgE-FcεRI signaling leads to histamine and cytokine release, causing airway inflammation and bronchoconstriction.
3. Eosinophil Recruitment and Late-Phase Asthma: IL-5 (Gene: 3567) and IL-3 (Gene: 3562) facilitate eosinophil activation, leading to airway damage. CCL11 (Gene: 6356, also known as eotaxin) recruit's eosinophils to inflamed lung tissue. Eosinophilic toxic mediators such as PRG2, RNASE3, and EPX contribute to airway inflammation and tissue damage.
4. Inflammatory Regulation and Immunosuppression: IL-9 (Gene: 3578) and TNF (Gene: 7124) drive inflammation, worsening asthma symptoms. IL-10 (Gene: 3586) is an anti-inflammatory cytokine that modulates excessive immune responses, though its role in asthma remains complex.

The table reinforces the strong link between IgE and asthma, showing how IgE production, mast cell activation, and eosinophilic inflammation drive disease progression.

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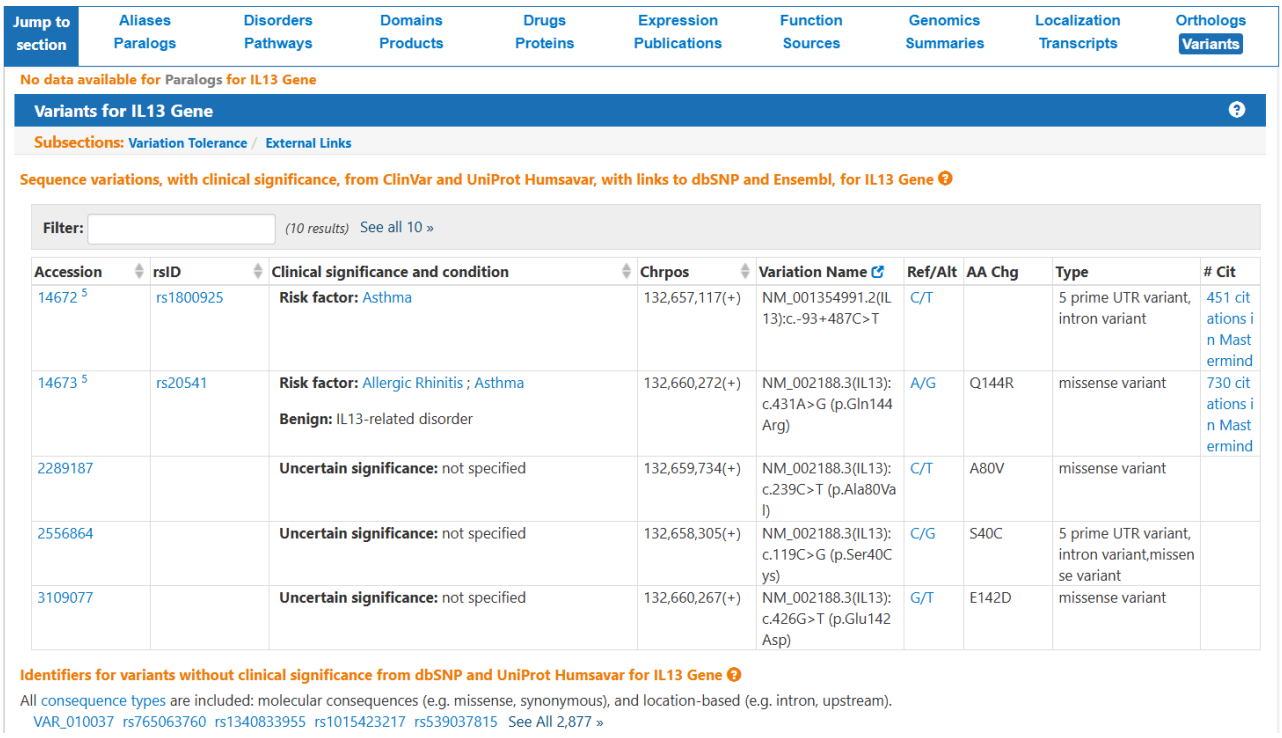
3.3. GeneCards Database Analysis



Jump to section | Aliases Paralogs | Disorders Pathways | Domains Products | Drugs Proteins | Expression Publications | Function Sources | Genomics Summaries | Localization Transcripts | Orthologs Variants

No data available for Paralogs for IL13 Gene

Variants for IL13 Gene

Subsections: Variation Tolerance / External Links

Sequence variations, with clinical significance, from ClinVar and UniProt Humsavar, with links to dbSNP and Ensembl, for IL13 Gene

Filter: (10 results) See all 10 »

Accession	rsID	Clinical significance and condition	Chrpos	Variation Name	Ref/Alt	AA Chg	Type	# Cit
14672 [5]	rs1800925	**Risk factor:** Asthma	132,657,117(+)	NM_001354991.2(IL13):c.-93+487C>T	C/T		5 prime UTR variant, intron variant	451 citations in Mastermind
14673 [5]	rs20541	**Risk factor:** Allergic Rhinitis ; Asthma **Benign:** IL13-related disorder	132,660,272(+)	NM_002188.3(IL13):c.431A>G (p.Gln144Arg)	A/G	Q144R	missense variant	730 citations in Mastermind
2289187		**Uncertain significance:** not specified	132,659,734(+)	NM_002188.3(IL13):c.239C>T (p.Ala80Val)	C/T	A80V	missense variant	
2556864		**Uncertain significance:** not specified	132,658,305(+)	NM_002188.3(IL13):c.119C>G (p.Ser40Cys)	C/G	S40C	5 prime UTR variant, intron variant,missense variant	
3109077		**Uncertain significance:** not specified	132,660,267(+)	NM_002188.3(IL13):c.426G>T (p.Glu142Asp)	G/T	E142D	missense variant	

Identifiers for variants without clinical significance from dbSNP and UniProt Humsavar for IL13 Gene

All consequence types are included: molecular consequences (e.g. missense, synonymous), and location-based (e.g. intron, upstream).
VAR_010037 rs765063760 rs1340833955 rs1015423217 rs539037815 See All 2,877 »

Figure 3: Genecards Data Analysis of IL-13 gene variants. (Source: Genecards)

The image displays a table of IL-13 gene variants extracted from a genomic database, highlighting their clinical significance, genetic variations, and associated conditions. Several IL-13 variants are linked to asthma and allergic disorders, reinforcing its role in IgE regulation and airway inflammation. The table lists different sequence variants of the IL13 gene along with their clinical significance, chromosomal positions, variation names, and mutation types (Fig.3).

Some variants are explicitly associated with asthma and allergic conditions, while others are classified as having 'Uncertain significance,' indicating that their clinical impact is not yet well established. Hence, the further analysis was done with the selected variants. The circled variants fall into this category and include:

- Variant at position 132,658,305 (C/G): Results in an amino acid change from Serine (S) to Cysteine (C) at position 40 (S40C). This variant is located in the 5' UTR and is also classified as a missense variant, which may influence protein function.
- Variant at position 132,660,267 (G/T): Leads to an amino acid change from Glutamic Acid (E) to Aspartic Acid (D) at position 142 (E142D). This is also a missense variant, meaning it alters the protein sequence and could potentially impact its function.

These amino acid substitutions have the potential to modify IL-13 protein activity, thereby affecting IgE-mediated responses and asthma pathogenesis.

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3.4. UniProt Protein Analysis:

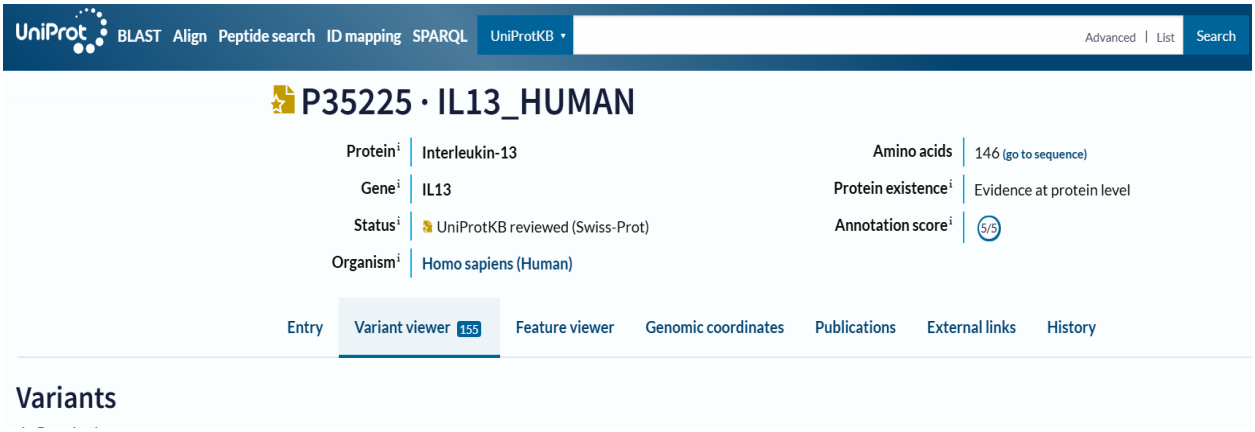
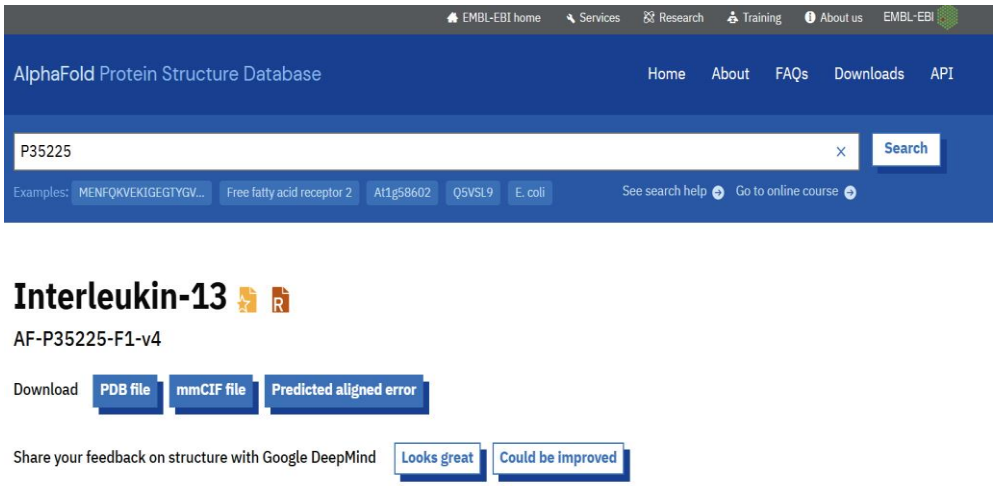


Figure 4: UniProt database entry for Interleukin-13 (IL-13) (Source: UniProt database)

The UniProt database entry for Interleukin-13 (IL-13) (UniProt ID: P35225) was analyzed to explore its role in allergic asthma and its correlation with IgE. (Fig 4) IL-13 is a key cytokine in the Th2 immune response, driving airway inflammation, mucus hypersecretion, and IgE production, all of which contribute to asthma pathogenesis. It is a 146-amino acid cytokine with confirmed protein-level evidence and a high annotation score (5/5), signifying well-validated functional data.

The UniProt Variant Viewer identified 155 genetic variants, some of which may impact IL-13 signaling and its receptor interactions, potentially influencing IgE-mediated responses in asthma. IL-13 regulates IgE class switching in B cells by signaling through the IL-4Rα/IL-13Rα1 receptor complex, which in turn activates the JAK-STAT6 pathway, a crucial mechanism in allergic inflammation. Genetic variations in IL13 may alter cytokine activity, modifying IgE levels and enhancing airway hyperresponsiveness, thereby affecting individual susceptibility to asthma. These findings underscore IL-13's pivotal role in IgE-mediated asthma pathogenesis. Further bioinformatics analyses using KEGG and DynaMut2 can provide deeper insights into IL-13 structural variations and their potential effects on asthma severity.

3.5. AlphaFold and RCSB PDB protein data banks:



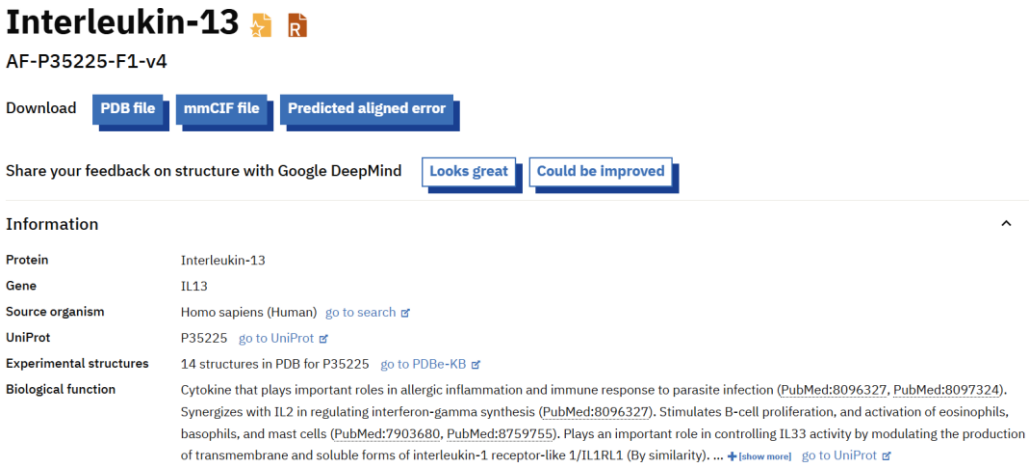


Figure 5(A&B) : AlphaFold and RCSB PDB protein data bank for IL-13 structural and functional analysis (Source: AlphaFold and RCSB PDB protein data bank)

The structural and functional analysis of Interleukin-13 (IL-13) (AF-P35225-F1-v4) using AlphaFold and UniProt provides critical insights into its role in allergic asthma and IgE regulation. (Fig. 5 A&B) IL-13 is a key Th2 cytokine involved in allergic inflammation, driving B-cell proliferation, eosinophil activation, and IgE class switching, all of which contribute to asthma pathogenesis. Encoded by the IL13 gene in Homo sapiens, IL-13 has 14 experimentally determined structures available in the Protein Data Bank (PDB). Structural modeling by AlphaFold enhances the understanding of its folding dynamics and receptor interactions.

Functionally, IL-13 synergizes with IL-2 to regulate interferon-gamma synthesis, influencing immune cell responses. It plays a direct role in B-cell activation and IgE production, exacerbating airway hyperresponsiveness in asthma. Additionally, IL-13 modulates IL-33 activity, amplifying allergic inflammation. Genetic variations in IL13 can alter protein function, impacting IgE production and asthma severity. Increased IL-13 expression has been linked to elevated serum IgE levels, which enhance mast cell activation and histamine release, further worsening asthma symptoms.

3.6. DynaMut2 Structural and Stability Analysis:

Two variants, NM_002188.3(IL13) ‘p.Ser40Cys’ and ‘p.Glu142Asp’ (A&B) were selected based on the results from Uniprot and AlphaFold databases, which demonstrated missense mutations and could lead to amino acid changes resulting in structural impact on signaling functions and could contribute to disease severity.

(A) VARIANT - NM_002188.3(IL13) – (p.Ser40Cys)

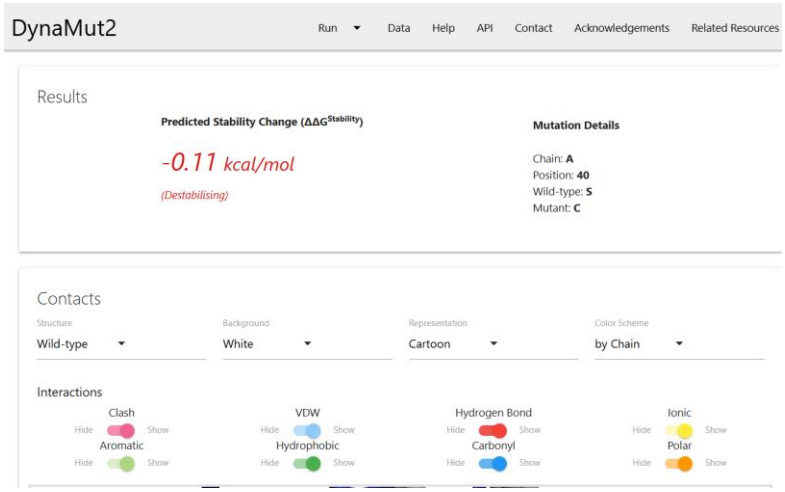


Figure 6 (A): DynaMut2 analysis for Interleukin-13 for the variant NM_002188.3(IL13) – (pSer40Cys) (Source: DynaMut2)

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Predicted Stability Change -

ΔΔG Stability Change: -0.11 kcal/mol

A negative ΔΔG value suggests that the mutation from Serine (Ser40) to Cysteine (Cys40) slightly destabilizes the protein structure (Fig.6(A)). However, the magnitude of the change is small, indicating a minimal effect on protein stability.

WILD TYPE (Ser40) MUTATED (Cys40)

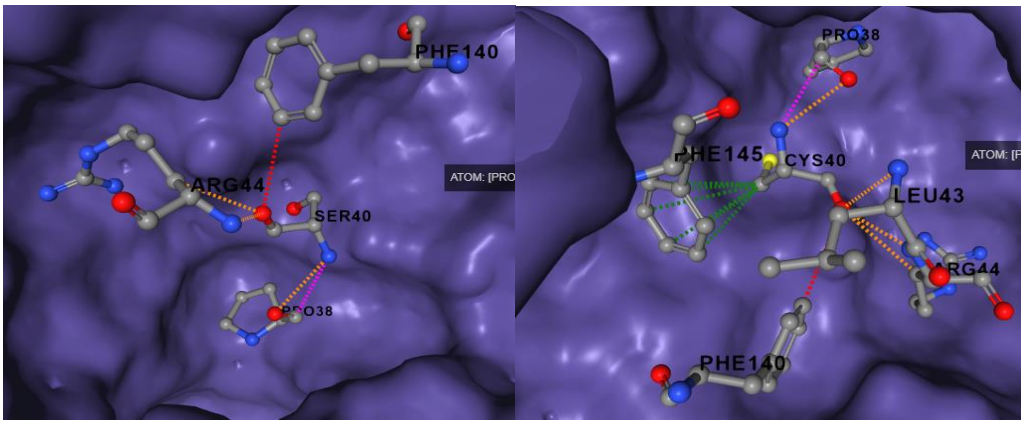

Figure 6(B): Structural comparison of (pSer40Cys) Wild-Type vs. Mutant the DynaMut2 tool. The different colors represent the various types of bonds present in the protein structure. (Source: DynaMut2)

Wild-Type (Ser40)- Hydrogen bonds are observed, contributing to stability. Interaction with Arg44 and Pro38 is present. The side chain of Ser40 engages in hydrogen bonding due to its hydroxyl (-OH) group (Fig.6(B)).

Mutant (Cys40)- New interactions appear involving Leu43, Phe145, and Arg44. The Cysteine (-SH) group may alter bonding patterns. A possible loss of hydrogen bonding compared to Ser40, contributing to the slight destabilization (Fig 6(B)).

The negative ΔΔG value (-0.11 kcal/mol) suggests that S40C is destabilizing, possibly affecting protein stability or function. If this region plays a crucial role in IgE-mediated signaling, the mutation could influence protein-protein interactions, potentially affecting its involvement in asthma-related pathways.

(B)) VARIANT - NM_002188.3(IL13) – (p.Glu142Asp)

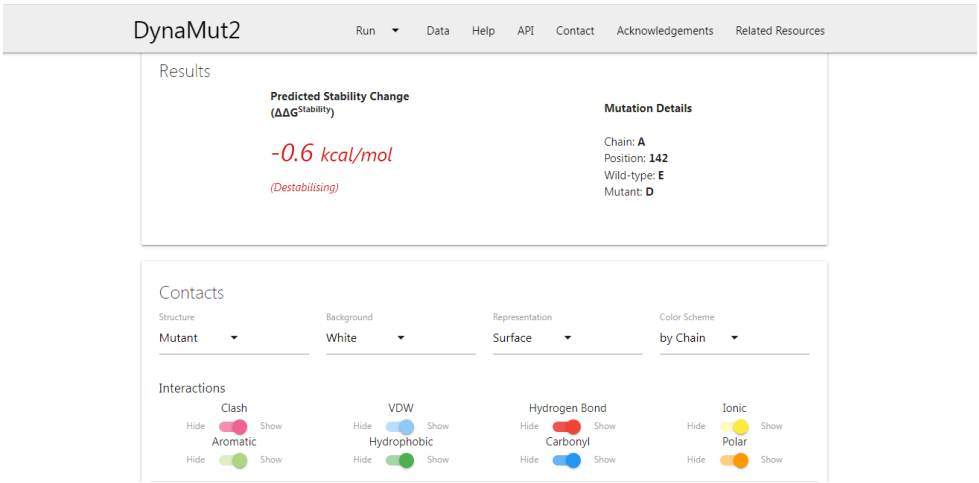

Figure 6(C): DynaMut2 analysis for Interleukin-13 for the variant NM_002188.3(IL13) – (pGlu142Asp) (Source: DynaMut2)

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Predicted Stability Change -The mutation analyzed is E142D, meaning glutamic acid (E) at position 142 has been substituted with aspartic acid (D) (Fig.6(C)) . The Predicted Stability Change (ΔΔG Stability) is – **0.6 kcal/mol**, indicating that this mutation is destabilizing the protein structure, though the magnitude of destabilization is moderate.

WILD TYPE (E142) MUTATED (A142)

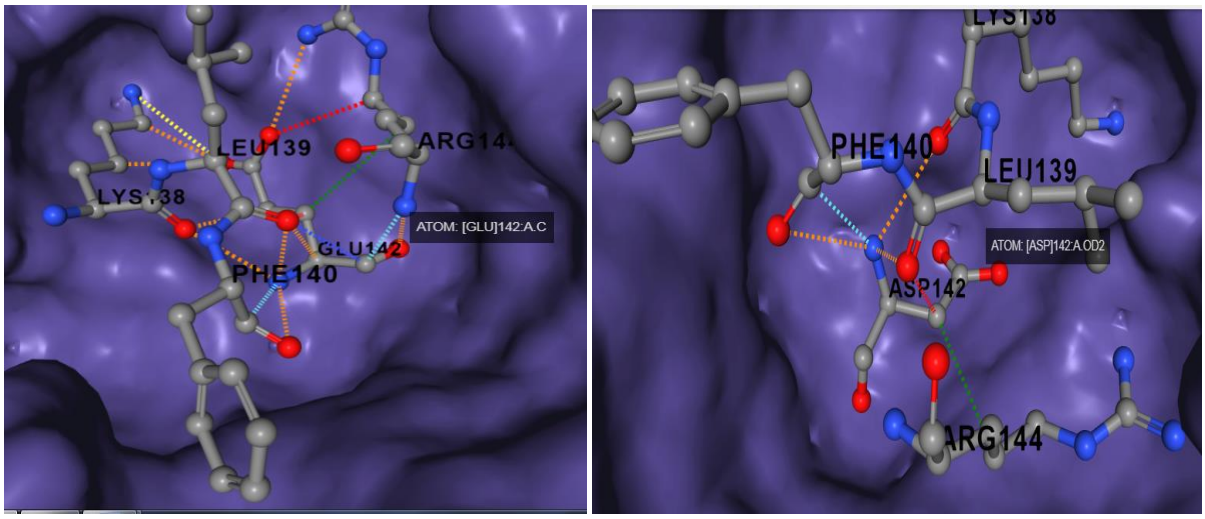

Figure 6(D): Structural Comparison (pGlu142Asp) Wild-Type vs. Mutant by the DynaMut2 tool. The different colors represent the various types of bonds present in the protein structure. (Source: DynaMut2)

Structural Comparison (Wild-Type vs. Mutant)

Wild-Type (Glu142)- Glutamic acid (E142) forms interactions with nearby residues LEU139, PHE140, ARG144, and others. The presence of hydrogen bonds (red dashed lines) (Fig. 6(D)) and other molecular interactions suggests a stable local environment.

Mutated (Asp142)- The substitution of glutamic acid with aspartic acid at position 142 leads to different interactions. There is an observable change in molecular contacts, with differences in hydrogen bonding and spatial orientation. (Fig. 6(D)) The structural representation suggests a potential loss or alteration of key stabilizing interactions.

The negative ΔΔG value (-0.6 kcal/mol) suggests a moderate destabilizing effect, which might impact the protein's function. Depending on the role of this region in the IgE-associated protein, this mutation could influence its binding affinity, structural integrity, or functional activity. If this protein is crucial for asthma pathophysiology, this mutation may have implications in disease susceptibility or severity.

4. DISCUSSION

Immunoglobulin E (IgE) is an antibody that binds to mast cells and basophils upon allergen exposure, playing a key role in asthma exacerbations. These exacerbations lead to episodes of wheezing, coughing, difficulty in breathing, and bronchial constriction, significantly impairing quality of life and contributing to high healthcare costs[15]. IgE antibodies are most widely recognized for their function as mediators of the allergic response, which can result in asthma in its most severe forms. A variety of symptoms, from mild to severe and potentially fatal, can be associated with IgE-mediated allergic reactions[16]. This study aimed to analyze the role of a gene associated with IgE and asthma by evaluating its genetic variants and their potential structural and functional impacts using bioinformatics approaches.

Our findings identified two key variants within the selected gene: E142D and S40C. Computational analysis using DynaMut2 provided insights into the structural and stability implications of these variants. The E142D variant exhibited minor destabilizing effects on the protein, potentially affecting its binding interactions. The decrease in stability, as predicted by DynaMut2, suggests that this substitution might influence protein function, possibly altering its role in IgE regulation and asthma pathogenesis. Previous studies have linked amino acid substitutions in immune-related genes to functional disruptions that exacerbate inflammatory responses in allergic diseases. Therefore, the presence of the E142D variant may contribute to individual susceptibility to asthma through its effects on protein stability and interaction dynamics.

Similarly, the S40C variant was analyzed for its potential impact on protein structure and function. The substitution of serine with cysteine introduces a thiol (-SH) group, which can lead to aberrant disulfide bond formation, potentially altering protein folding and stability. Our results indicate that S40C may induce conformational changes, which could influence the protein's role in IgE-mediated immune responses. Studies have demonstrated that cysteine substitutions can affect protein-protein interactions, particularly in immune system components, thereby modulating immune activation and inflammation. Given the crucial role of IgE in asthma pathogenesis, even subtle alterations in protein structure may have significant functional consequences.

Beyond structural effects, these genetic variants may influence gene expression levels, post-translational modifications, and overall immune signaling pathways. Variants that reduce protein stability could lead to protein degradation, loss of function, or compensatory mechanisms that exacerbate asthma symptoms. While our computational predictions suggest destabilizing effects for both E142D and S40C, further experimental validation is required to confirm their physiological relevance.

A novel variant in the coding region of the IL13 gene was identified and found to be significantly associated with elevated IgE levels and atopic dermatitis (AD) in both genotypic and allelic association analyses within a German study population[17]. In allergic asthma patients, IgE production appears to be more dependent on IL-13 compared to non-atopic individuals, likely due to both increased IL-13 expression and an enhanced IgE response to IL-13 stimulation[18]. Thus, indicating that IL-13 promotes IgE production and IgE-based mucosal inflammation leading to the atopic phenotypes, suggesting IL-13 cytokine as a central cytokine in promoting asthma[19]. This study highlights the important role of genetic variants in the IL-13 gene in affecting IgE levels and their possible link to asthma risk. By studying specific mutations and using DynaMut2 to assess their effect on protein stability, we identified structural changes that may play a role in asthma development. These findings improve our understanding of the genetic factors behind asthma and could help in creating targeted treatments or personalized therapies for better asthma management.

5. CONCLUSION:

This study highlights the critical role of IL-13 and IgE in the pathogenesis of allergic asthma, emphasizing their involvement in immune regulation and inflammatory responses. Through bioinformatics analysis, we identified key molecular interactions, signaling pathways, and structural variations that may contribute to disease progression. These findings enhance our understanding of the underlying mechanisms of asthma and provide a foundation for future research aimed at developing targeted therapeutic strategies. While further experimental validation is necessary, this study offers valuable insights that may support advancements in personalized approaches to asthma management.

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Conflict of interest declaration

The authors declare that there are no conflicts of interest.

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