CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

In Silico Analysis of Multimorbidity between Asthma, Rhinosinusitis, Sinusitis and Gastroesophageal Reflux Diseases

by

Sumbal Shahzadi

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2021

My dissertation work is devoted to My Family, My Teachers and My Friends. I have a special feeling of gratitude for my beloved family. Special thanks to my supervisor, Dr. Sahar Fazal whose uncountable confidence and guidance enabled me to reach this milestone.

CERTIFICATE OF APPROVAL

In Silico Analysis of Multimorbidity between Asthma, Rhinosinusitis, Sinusitis and Gastroesophageal Reflux Diseases

by

Sumbal Shahzadi

(MBS191020)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Mazhar Qayyum	PMAS, UAA, Rawalpindi
(b)	Internal Examiner	Dr. Shaukat Iqbal Malik	CUST, Islamabad
(c)	Supervisor	Dr. Sahar Fazal	CUST, Islamabad

Dr. Sahar Fazal
Thesis Supervisor
April, 2021

Dr. Sahar Fazal
Head
Dept. of Bioinformatics and Biosciences
April, 2021

Dr. Muhammad Abdul Qadir
Dean
Faculty of Health and Life Sciences
April, 2021

Author's Declaration

I, **Sumbal Shahzadi** hereby state that my MS thesis titled "**In Silico Analysis of Multimorbidity between Asthma, Rhinosinusitis, Sinusitis and Gastroesophageal Reflux Diseases**" is my own work and has not been submitted previously by me for taking any degree from Capital University of Science and Technology, Islamabad or anywhere else in the country/abroad.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw my MS Degree.

(Sumbal Shahzadi)
Registration No: MBS191020

Plagiarism Undertaking

I solemnly declare that research work presented in this thesis titled "**In Silico Analysis of Multimorbidity between Asthma, Rhinosinusitis, Sinusitis and Gastroesophageal Reflux Diseases**" is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Capital University of Science and Technology towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS Degree, the University reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized work.

(Sumbal Shahzadi)
Registration No: MBS191020

Acknowledgement

"And your God is one God. There is no deity [worthy of worship] except Him, the Entirely Merciful, the Especially Merciful" [2:163]. First and foremost, I wish to say thanks to **Allah** (S.W.T) for giving me blessings, power and knowledge to finish this research. Secondly, I wish to express my gratitude to my supervisor **Dr. Sahar Fazal** for her help, precious time and supervision. I pay my thanks to her sincerely for her assistance, motivation and advice in this field of research. She helped me from the understanding of this subject till the write up of final thesis. I am deeply grateful to **my family and my parents** for their support and encouragement till the end of my MS thesis. Their prayers and guidance have led me here.

(Sumbal Shahzadi)

Abstract

The coexistence mechanics of asthma, sinusitis, rhinosinusitis and reflux gastroesophageal disease are largely unknown. Rhinosinusitis, sinusitis, and gastroesophageal reflux disease are the most commonly recorded reasons for comorbid asthma. In silico study was conducted to describe the molecular mechanisms of the comorbidity of asthma with sinusitis, rhinosinusitis and gastroesophageal reflux disease. A list of associated genes were retrieved from polysearch, uniport and ensemble and find common genes for selected diseases. Selected common genes (HLA-A and HLA-B) were used to retrieved the related pathways from KEGG. Proteins related to the targeted genes were screened manually from HIPPIE and InnateDB and also from pathways in HLA-A gene there are KIR3DL2, KIR3DS1 and STAT3 proteins are common. In HLA-B there are B2M, CD8A, LILRB1, LILRB2, EZR, MSN, PL53, PSMD1, PAICS, RUUBLI, AHCY, VCP, TRIM28, ADRB2, ARHGEF4, EDEM1, HSPA5, NEDD8, PCK1, STAT1, SUM01, TRIB3 and UBD proteins are common. Protein-Protein interaction network was generated using bio-intomics and funcoup, from network predicted proteins are PSMB6, PSMB2, RPL3, HSPA8, NME2, PCNA, TCP1, KPNB1, UBA1, ATP5F1A, ENO1, EEF2, ATP5F1B, TUBB, NED, RACK1, ACTB, RPS27, RPS4X, RPSA, EEF1A1, GAT1, PPP2B1A, RPSA, DNAJA1, GP1, TUBB, PARVA, PARVB, PXN, LIMS2, LIMS1, RSU1, LASP1, CRK, PDLIM5, TWF2, RAC1, RPS9, RPL7 and CNDP2. Functional annotation analysis was carried out using David tool. HLA-A and HLA-B functional enrichment analysis revealed from the common proteins. In HLA-A annotation cluster significant value indicate that these proteins are not clustered accidentally rather they are significantly more enriched. In HLA-B functional annotation 6 clusters are significant (cluster 1, 2, 3, 4, 5 and 7).

Contents

List of Figures

List of Tables

Abbreviations

ABPA	Allergic brinchopulmonary aspergillosis
ADHD	Attention-deficit hyperactivity disorder
ADIPOR1	Adiponectin activates adiponectin receptor 1
CERNAS	Competing endogenous RNAS
COPD	Chronic obstructive pulmonary disease
CRS	Chronic rhinosinusitis
CT	Computerized tomography
CVD	Cardiovascular disease
CYSLTS	Cysteinyl leukotrienes
ECM	Extracellular Matrix
GERD	Gastroesophageal reflux disease
GWAS	Genome-wide analysis studies
HIPPIE	Integrating Protein Interaction Networks with Experiment
HO-1	Heme oxygenase
INOS	Inducible gas synthase
KEGG	Kyoto Encyclopedia of Genes and Genomes
LNCRNAS	Long noncoding RNAS
MHC	Major histocompatibility complex
NQO1	NADPH quinone oxidoreductase 1
OSA	Obstructive sleep apnea
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
TLRS	Thymic stromal lymphopoietin
TPA	Tissue plasmanogen activator

Symbols

α	alpha
β	beta
γ	gamma
κ	kappa
μ	mu

Chapter 1

Introduction

Asthma is characterized as an inflammatory disorder that is chronic. Sporadic respiratory difficulties, such as wheezing and coughing when exposed to stimuli, such as allergens, cold weather and exercise, are the usual clinical symptoms of asthma. It has a distinct complexity between people and even within the individual. It is referred to as allergic asthma when asthma is activated by an immunological response to an allergen, whereas it is referred as the non-allergic asthma when this is caused, for example, through physical activity. In certain cases, IgE antibodies and the release of histamines cause an asthma attack, but it may also be a case that the attack is caused by a non-allergic action as a result of cold temperatures, although both forms have airway inflammation. Clinically, allergic and non-allergic asthma are also used as diagnostic types of asthma. It is also common for those with allergic asthma to have non-asthmatic reactions, such as stress [1]. Some researchers have shown that non-allergic asthma is serious because of lower controls than its allergic form [2]. Asthma is characterized in four areas, none being a mandatory diagnostic criterion: symptoms, obstruction of airways, respiratory hyperresponsiveness and inflammation of the airway [3]. In Sweden, about 8.3–12% of the population is estimated to be affected [4].

Patients with irregular lung sound and breathing inflammation are usually affected. Asthma observations including wheezing, high breath and skin rashes, which are believed to be caused through allergens [5]. During medical history

collection, more signs of asthma may be determined. The specialist will collect data on the extent and severity of asthmatic signs (e.g., wheezing and breathing shortages) as well as whether the presence or degradation of any allergens and events. If medical history and physical exams support a diagnosis of asthma, tests for the lungs have probably follow [6] .

1.1 Pathogenesis

Although the actual cause of asthma is not known, many studies have made it possible to understand certain essential molecular bases of the condition. The Asthma stem pathogenesis arises from various diverse levels of the airway which can function individually or together with a different stage. The airway epithelium is probably the most critical stage of pathophysiology. This epithelial layer is a primary protection in healthy people, by trapping allergens in the mucus and transferring mucus and trapped allergens from the breathing system using their cilia. The epithelial layer must maintain strong bonds in order to perform this role. However, these close junctures are broken in individuals who suffer from asthma, allowing pathogens to enter the body and become sensitized. This disturbance leads to allergies to penetrating allergens that thus cause an inflammatory response. In particular, eosinophilic recruitment leads to cytokines released (a form of immune response-involved cell signaling protein) that contribute to asthma inflammation of airways [7].

1.2 Symptoms

Asthma symptoms vary significantly between patients and degree of severity. Asthma's most common symptoms include short breath, a breath with a slight whisper (wheezing), coughing, which appears to intensify the tightness of the chest and lying down [6]. If the symptoms get worse, they can lead to sleeping difficulties. During asthma attacks, more severe symptoms are seen which are clearly described as increased swelling severity and inflammation of the air

passages. These events normally lead to respiratory viruses, allergens to the atmosphere (e.g., ozone or pollen) and physical exercise. Treatment can range from inhaler use to 3 emergency treatments for these incidents. Increased occurrence of asthma attacks and the severity of the symptoms indicates overall progressive asthma deterioration [8].

1.3 Asthma and Allergy

Allergy and asthma are general diseases that are in touch with almost everyone in society. Asthma and allergy have traditionally been treated as merely psychosomatic disorders owing to their emotional distress or mental agitation [9, 10]. Asthma was seen in the 1900s as an organic condition when corticosteroid therapy dramatically changed the life of the person who was sick, changing the understanding about both asthma and allergy in general. Although recognized as life-threatening, asthma was partially regarded before the 1960s as a disorder restricted to socially and education, and it became recognized later as affecting all society groups and posing a serious threat to overall health and life, especially in the poor parts of society [11]. Later in the years 1968 IgE confirmation of the previous predictions of the fifth immunoglobulin that an allergic reaction is causing the atopic disease. This announcement leads to a deeper understanding, more useful therapies, and shifts the perception of allergic diseases to what we now know [2, 12].

The researchers have focused on psychological factors, from coexistence and multimorbidity in asthma/allergy with intolerances to chemical compounds and buildings, knowledge of different form of discomfort in various asthma diagnoses and allergies, and use levels of social support and coping strategies. The four most popular types of allergy and asthma diagnosis were viewed in a broad perspective. For centuries, asthma and allergy have fascinated scientists in aspects from underlying causes to prevention. Initial documentation from 2500 BC for various types of allergic disease were found. The asthma clinical description is from ancient times [13] where early theories indicated that auto-regulation of anger could

prevent asthma attacks [14].

The Jewish rabbi, doctor and philosopher who lived in Egypt, Maimonides (1135-1204) suggested psychosomatic asthma. On the one hand, a disordered psyche can influence an individual's somatic or physical well-being. On the other hand, happiness might have beneficial consequences, as the heart is thought to be glad and to stimulate the blood and mental function. Allergic disorder has progressed over time into various pathophysiological syndromes. Inflammation may more or less be localized and in reaction to relatively harmless external irritants such as allergens is one of the most critical aspects. In recent decades, the change in asthma awareness and allergy can be reflected in the way it has been handled [15].

Asthma was seen mainly during the second half of the 19th century as a psychosomatic condition, where emotional stress and nervous system imbalances were seen as its main etiological causes. This was the main clinical technique for asthma to alleviate anxiety [11]. This can be compared to the current asthma/allergy view, which is primarily seen as inflammatory diseases, in which medication such as corticosteroids and histamine treat both inflammation and allergic reactions [10]. Once the IgE is an inflammatory disorder mediator [16]. The production and enhancement of the daily lives of beneficial medications such as corticosteroids and inhalation formulations enabled people to manage their allergic disease [17]. The treatment of asthma, and particularly the nonallergic, was improved during the early 1970s by inhaled corticosteroids. With inhaled corticosteroids, adverse effects of orally given cortisone may now be avoided. Asthma was later confirmed in the 1990s as an inflammatory disorder that may explain the beneficial impacts of anti-inflammatory agents. Psychoneuroimmunology (PNI) is the relation between immunology and psychology. The correlation lies in the ties between psychologically and biologically plausible theories of how psychological factors can affect immune systems and the immune system-mediated disease. These concerns have inspired this study, considering that it is more a disease than a biomedical perspective. Asthma and allergy prevalence and properties Although findings are conflicting [4], A rising number of people worldwide, in particular developed countries, tend to suffer from allergy and asthma [18]. Ekerljung and colleagues (2010)

have come to the conclusion that this increased prevalence may be an indicator that the prevalent asthma has not changed over recent years, but that the improvements in diagnostic procedures may not necessarily increase the prevalence. The lowest prevalence in the Mediterranean and the highest prevalences in Great Britain and Scandinavia are projected to be 5-10% in Europe [19]. Around 300 million the world's population is estimated to have asthma, and by 2025 the number of people is estimated to have risen to 400 million [20]. Asthma and allergy have both been documented to affect quality of life aspects (QoL) [21] to emotional suffering [22], Where stress can lead to a copy result, this can in turn build a negative loop [23], but also to the elevated risk of disease [24]. The negative aspects of diseases can lead, in combination, to a decrease in the financial security of the person because of days lost in work/school and three restrictions in daily social life. The need for medical treatment and hospitalization often cost society asthma and allergy [24]. It was proposed that production loss in Sweden alone is projected at £2,7 billion per year due to sick days of allergic rhinitis and common cold. This indicates that both asthma and allergy diseases are illuminated from different angles in order to find differences to mitigate disease and to improve their QoL in these people [25].

1.4 Asthma Genetic Research in Pakistan

Asthma is a complex miscellaneous and polygenic disorder and therefore more than 100 genes have been identified to be asthma related. In Pakistan, some research on genetics of Asthma have recently been published, writing a genetic relationship of CD-14 genes with SNP markers [26], IL-4 [27], ADAM33 [28], IL-13 [29] and 17q21 [30, 31]. Such studies have been carried out on a very small scale and do not provide sufficient proof of the genetics related to the disease. Consequently, there is an enormous scope and desire for Pakistani population to discover asthma genetics. This will allow us to gain accurate knowledge which can be useful later for better diagnosis and therapy of this disease. Inflammation and immunity response pathways are implicated in many asthma-susceptible cytokine genes in Table 1.1 [32].

TABLE 1.1: Cytokines genes associated with Asthma which is involved in inflammatory and immune response pathways.

No of Genes	Genes	Chromosomes	Molecular Functions	Variants	References
1	CC16	11q12.3	Secretoglobin family 1a member 1	38a/g	[33]
2	CCL11	17q12	CCR chemokine receptor binding	-384a/g	[34]
3	CCL5	17q12	Phosphatidylinositol phospholipase c activity; Chemokine receptor binding	-403g/a -28c/g	[35]
4	CSF2	5q31.1	colony-stimulating factor receptor binding;	Rs25882	[36]
5	IFNG	12q15	Interferon-gamma receptor binding;	874a/t	[37]
6	IL10	1q32.1	Interleukin-10 receptor binding;	-1082a/g	[38]
7	IL12B	5q33.3	Cytokine receptor activity	Rs3212227 Rs20541	[39]
8	IL13	5q31.1	Interleukin-13 receptor binding	rs848 rs1295686	[40]
9	IL1B	2q14.1	Interleukin-1 receptor binding	Rs16944 rs1143634 Rs928413	[39]
10	IL33	9p24.1	Protein binding	Rs3939286 rs1342326	[41, 42]

11	IL4	5q31.1	Interleukin-4 receptor binding; Growth factor activity	-589c/t 33c/t	[43, 44]
12	IL5	5q31.1	Interleukin-5 receptor binding; growth factor activity	-703c/t	[45]
13	IL6	7p15.3	Interleukin-6 receptor binding; Growth factor activity	-174g/c rs1800795	[46]
14	LTA	6p21.33	Tumor necrosis factor receptor binding	NCOI	[47]
15	MIF	22q11.23	Cytokine receptor binding	-173g/c	[47]
16	STAT6	12q13.3	Nucleic acid binding; transcription factor DNA binding transcription factor activity, sequence-specific DNA binding	rs167769 rs71802646	[48]
17	TNF	6p21.33	Tumor necrosis factor receptor binding	-308g/a -238g/a Rs1837253	[39]
18	TSLP	5q22.1	Cytokine activity	rs3806933 rs2289276	[49]

1.5 Problem Statement

The effect of comorbid conditions on asthma pathophysiology and their influence on the management of asthma or modulation of treatment solutions is highly concerned. This is especially important because significant determinants of asthma phenotypes increasingly identify comorbid conditions, and their associations have to be identified with comorbidities in terms of genetics.

1.6 Aim of Study

This study aims to identify asthma-associated multimorbidity genes and pathways shared by Sinusitis, Rhinosinusitis and Gastroesophageal reflux disease.

1.7 Objectives

To achieve the aim of the study, following objectives have been set,

1. To identify the proteins encoded by the asthma and comorbidities-associated genes.
2. To identify the pathways shared between diseases.
3. To construct functional interaction network of protein or gene (for characterization of interactions).
4. To find the crosstalk among the shared pathways.

1.8 Scope

Co-occurrence of two or more medical conditions within a person is more understanding of common mechanism and links of multi morbidities between asthma and other diseases can better help the researchers and physicians to destine asthma management strategies and treatments.

Chapter 2

Literature Review

2.1 Epidemiology

The Asthma contributor, which makes up 1 percent of the global burden of disease, is a major contributor to global morbidity and mortality, and one of the world's most influential diseases. In addition, asthma leads to a loss of some 15 million life-years annually adjusted for disabilities. While the concept of asthma in the world is known, the accurate occurrence level is difficult to measure, because the description of asthma is very subjective in comparison with other disorders. The differences in these statistics are possibly attributable to the varying expressiveness and the considerable variation in patients' incidence of symptoms. In addition, asthma cases can be further divided into age of onset or incidents that increase the rate of asthma attacks (e.g., pre-adolescent versus late onset and exercise-induced versus obesity-related)[50]. Although reliable calculations may not be consistent, overall statistics indicate that approximately 1 in every 250 deaths worldwide is responsible for asthma. Although the incidence of asthma in all communities of countries affects individuals of all ages and socio-economic classes, it is not equally distributed. In the lower classes of Westernized, high-income countries, asthma tends to be more prevalent. In the United States, for example, asthma care costs about $56 billion in a single year. Other English-speaking countries, such as the UK, Australia and the UK are much more prevalent than countries in

the Mediterranean and Eastern Europe, such as Greece and Uzbekistan (as large as 36.8 percent) (as low as 1.6 percent) [50]. In the United States, there are several racial inequalities. For example, asthma is higher among African Americans and South Asians. As these sub-population's frequencies do not equate in the United States with those in their native countries, the ethnic differences are more unlikely to be due to genetics. Rather, it was suggested that for socioeconomic reasons, asthma occurs more commonly in Afro-Americans and South-Americans. These communities, for example, appear to have low incomes and have a lower risk of getting adequate access to asthma care, thus causing them to resort to emergency treatment. In contrast with other dailies such as food protection and shelter, this situation is often seen among low-income communities. At different stages of life, asthma also displays different frequencies between the sexes. Pre-adolescent asthma, for instance, affects more men than women. In fact, males were hospitalized twice as often as females in the 0-10 age group. The most common chronic childhood condition is this initiation type of asthma. Asthma has important and wide consequences in the fact that asthma-related hospitalizations also have a direct impact on primary school attendance, as well as on children's medication obligations. Especially, pre-adolescent complications of 5-year-old asthma peak. Asthma affects more women than men, however, including all ages of inception. In particular, women were hospitalized about twice as often than men for the age group of approximately 30-60. Since the reversal of gender prevalence changes during puberty, these frequencies are due to sex hormones. However, the comparatively smaller size of female airway compared to that of males has been postulated [51]. Recent times also raised the incidence of asthma diagnosis. The risk associated with those born after 1966 is precisely double increased. Hospitalized individuals in the United States rose from 1980 to 1999 by 1.4 percent as a result of asthma attacks. At the same time, the mortality associated with US asthma has also increased (of 3.4 percent) [1]. It is estimated that by 2025 100 million more people are affected by asthma, at the current occurrence rate. Genetics More than 120 genes have been reported for connection with either asthma susceptibility or associated symptoms in peer-visited journals,

according to a systematic report from 2006 [52]. Through their interactions in the asthmatic pathway, these 120 established genes can then be organized into different subcategories: innate immunity, T helper cell differentiation, epithelial and mucosal immunity, and lungs and airway function. The majority of these 120 genes were identified with either a candidate gene strategy or positions. While candidate gene strategy calls for preliminary gene 6 information, position cloning approaches are basically a genome scan that compares the genotypes of asthma-affected people with non-affected individuals and thus does not require previous gene knowledge [53]. Most of these genes found had proteins that either operate within the plasma membrane or are secreted and inflammatory. Vitamin D receptor, G-protein A receptor (GPRA) and ADAM33 are three of the most well recognized and strongly accepted genes involved in asthma. ADAM33 was also one of the first genes known to be associated with asthma. The receptor for vitamin D is highly versatile because it regulates the functions of many genes, especially the immune system operators. Many proofs have been given that asthma plays a key role in the vitamin D recipient. Indeed, mouse and people who do not have the vitamin D receptor gene cannot engage in any inflammatory response. They are also completely asthma prone. The asthma vulnerability is also caused by the GPRA gene. Sadly, asthma genes are highly difficult to check for. This is due not only to the incoherence of asthma diagnosis but also to the very difficult of asthma pathophysiology. As the genetic causes of asthma are also highly variable, since asthma may come from various pathways. When potential effects of 7 gene interaction apply, this concept becomes much more complicated [52]. Many gene regions with asthma have shown to consist of more than one loci and this results in a susceptibility-enhancing additive effect. In addition, the quest for genetic connections to asthma wasn't in vain—it brought about a deeper understanding of the condition and even new hypotheses on asthma pathology [53]. In general, a rise in asthma incidence is closely linked to a Westernized urban lifestyle. The climate includes diet, early-life exposure to illness, home heating systems and exercise. The incidence in the English-speaking world in relation to Mediterranean countries is far higher, as discussed in 'epidemiology.' One of the big factors is the

diet. As far as lipids are concerned, Westernized diets usually contain far more Omega-6 fats than Mediterranean diets, which are more central to Omega-3 fats. Fats containing omega-6 are polyunsaturated fatty acids commonly found in food, such as vegetable oil and margarine. They support asthma because they appear to increase the risk of inflammatory reactions within the body. The opposite effects of omega-3 fats, usually found in oily fish, on the inflammatory system are observed. They are said to be anti-inflammatory and to help the concept of treating asthma [1] In addition, the rise in the occurrence of asthma in more recent times may be attributed to urbanization and technological development. In the past, for example, the majority of domestic heating systems relied on coal or wood. But household heating systems have switched to energy sources including gas and electricity in modern times. As energy sources have made this big revolution, the incidence of asthma has also increased. Other innovations and luxuries, such as automobiles, have also led to the growing prevalence of asthma [54]. Sulfur dioxide, nitrogen dioxide and ozone are other outdoor contaminants that raise the risk of asthma. While these external contaminants can easily be recalled indoors, the production of asthma still relies heavily on internal pollutants. For example, indoor contaminants like molds and pet dander as well as organisms that can infect the house, such as cockroaches and rodents, contribute significantly to an allergic climate, particularly damaging for those already susceptible to asthma [55].

2.2 Asthma Risk Factors

A risk factor is considered to raise the risk of developing asthma and allergy, for example, while a trigger factor is considered to intensify symptoms in already sensitized people. But between the risk factors and causes, there is some overlap. Smoking, for instance, raises asthma risk [56, 57], and smoking both primarily and secondarily can cause an acute exacerbation [58–60]. The trigger factors can in this regard be described as environmental, substantial and/or event factors that increase a person's risk for severe asthma deterioration or leading to the incidence or worsening of asthma symptoms lasting several hours or days [61]. In previous research, the risk factor for asthma development was found to be

allergy (e.g. atopic dermatitis and allergic rhinitis), obesity, social dependencies of age, urbanization, genetic and sex [57]. There have been many theories why asthma and allergy have evolved in various ways. Hygiene hypothesis is one of the most popular theories of the emergence of asthma and allergy. The report notes that an absence of exposure to infectious agents, symbiotic microorganisms (e.g., intestinal flora, probiotics) and pests increases their susceptibility to allergic diseases by preventing natural immune system growth. The absence of exposure is suspected to contribute to immune tolerance deficiencies [62]. In some cases, since risk and trigger factors can be the same, some triggers are stated to be more common than others. In a recent research, a broad selection of frequent cause 7 variables in their nearest environment have been found in asthma patients. This includes allergens, pollutants/irritants indoor/outdoor, strong scents, foods of various forms, weather, exercise, sinusitis/breath diseases, pressures, drugs and strong feelings that are seen to help the frequency and severity of the symptoms of asthma [63]. Exposure to cold weather, anxiety and stress are other conditions likely to cause non-allergic reactions [64].

2.2.1 Vitamin D

Detailed understanding has occurred during the past decade in the area of vitamin D for Asthma. Numerous studies have recommended that vitamin D deficiency could play a role in the severity of symptoms. It has been shown that vitamin D activation receptors maintain immunomodulatory and anti-inflammatory activities. Vitamin D receptor has been suggested to mitigate the effects of both adaptive and innate immunological responses by its influence on immune cells, dendritic cells and T and B lymphocytes. Through modifications within epithelial cells as well as alveolar macrophages, vitamin D could also play a role in airway inflammation and conduct pro-inflammatory cytokines transcription. Lack of Vitamin D in laboratory animals has resulted in an increase airway renovations, enhanced bronchial embolization of eosinophilia, decreased T cells, enhanced NF-KB expression, and improved pro - inflammatory cytokines [65]. In vitamin D lacking people, these have been hypothesized that similar systems can explain for Asthma. A series

of researches seem to have a correlation among vitamin D deficiency and adverse events of Asthma, such as poor pulmonary function, harmful symptoms, and more frequent exacerbations of Asthma. Consequently, poor parental vitamin D levels throughout pregnancy are correlated with greater wheezing incidence in infants. Animal studies have also shown that in embryo lung development, vitamin D plays an important role.

One research limited vitamin D through pregnancy and breastfeeding within the diet of rats. Changes in trachea contractility and communicating in the offspring of those rodents [65]. Vitamin D supplementation has been shown to act a remedy in a number of such mutations within the lungs of offspring in defective pregnant rodents [66]. Different results have been seen in the consequence of human researchers examined when vitamin D deficiency may raise a risk of developing Asthma [67]. In addition, genetic variations that influence either the amount of vitamin D and the reaction to treatment with vitamin D. Vitamin D may also enhance the variability throughout the results. In a comparative analysis of two human randomized trials, it was shown that maternal vitamin D supplements contributed in a decreased risk of asthma among the offspring at three years of age [68]. Different results have also been seen in human experimental studies that examined whether lack of vitamin D may result in much worse asthma outcomes among those with this disease. The new meta-analysis with individual study data showed that supplementation of vitamin D can minimize the pace for asthma exacerbation which involves systemic corticosteroids [69]. The vitamin D treatment, however, improves the response of the corticosteroid to asthma (VIDA), a trial of 408 asthmatic individuals, all of whom have had no effect upon this inhaled corticosteroid [70]. In secondary research, reduced service failures as well as exacerbations were observed for those inside the treatment for those who reached a level of 25 hydroxyvitamin D level of the minimum 30 ng/ml. Additional studies are required for clarification of vitamin D's function in asthma progression and treatment. In particular, the study should be specialized with in optimal dose or amount obtained, arguments for a vitamin D variable response and length of treatment in clinical studies.

2.2.2 Respiratory and Gastrointestinal Microbiota

The link between the microbiota and several cases of disease, as well as asthma and autoimmune disorders, recently have been rapidly discovered. Due to immunological variations promoted by revelation to muramic acid and endotoxin, outer membrane glycoproteins, and initiation to microorganisms in puberty is said to be protective. The incorporation of high levels and multiple classes of microorganisms contributes to improved activation of innate response, which is supposed to be ultimately contributed to the production of directing T cells and then to the elevation of oxidative stress. Recent experience has also been suggested to result in improvements in invariant pro - inflammatory cytokines [71]. Mouse models have shown that invariant natural killer cells multiply in fungus mice, presumably leading to increased inflammation and stronger allergic airway defenses [72]. European children with early farm coverage are now well studied to have lower asthma rates, possibly frequently exposed to different microorganisms [73]. Inhalation has been recommended because of the main mechanism for exposures, but oral exposure could also lead to a diverse microbiota as indicated in child eating asthmatic diseases [74]. However, other studies have shown that early asthma exposure in non-farming communities may also have a beneficial effect. That may be why the presence of people and animals inside the home is also associated with a lower risk of nuclear conditions in infants [75]. Because early exposures may help to take care of the diversity of microbiomes, for example, other exposures in young people may trigger microbial dysbiosis, antibiotic administration can cause long-term changes inside the intestinal microbiome. During caesarean delivery and caesarean section, intrapartum antibiotics were shown to result in variations in gastrointestinal pathogens that persisted for equal to 12 months post birth [76]. Observational studies have reported an association between early developmental exposures to antibiotic and therefore the production of allergies and asthma, while issues including infection intensities, antibiotic signals, and the type or category of antibiotics are having influence on early life exposures [77]. In comparison, breast feeding and delivery methods involve several early life parameters which impact the formation including its infant environment and are variously linked

to an asthma and allergic case [78]. The impact on the development of allergic asthma for feeding as well as routing are complex [79].

2.2.3 Tobacco Smoke And Asthma

Current reviews have shown that prenatal smoking can affect fetal lung growth and raise the chances of embryo asthma. There was a study that investigates people whose families are survivors to domestic violence and that the risk of asthma was increased if one or both older adults smoked. With paternal abstinence, the risk could be minimized, although the mothers preferred to smoke [80]. Uterine exposure to tobacco smoke, especially caffeine, leads to reduced fast decoupled load flow and decreased pulmonary obedience [81]. With passive smoking exposure in utero, improvements in DNA methylation, changes in a life form and a raise tendency designed for T helper 2 pathways also occur and are conjectured to contribute in increasing prevalence of Asthma [82]. Access to second-hand as well as third - hand cigarette smoke in infancy also increases the risk of emerging Asthma, it has been reported is that the risk of Asthma is about 20 percent. Post-natal passive smoking exposure can, distort pulmonary allergic reactions through TH2, increase inflammation. Hazard of autoimmune diseases, and exposure to infections, both of these are danger to develop Asthma simultaneously [83].

2.2.4 Air Pollution

Asthma development is credited to population growth and the resultant pollution needed to be, equally take part in the progress of asthma. A research in Europe got 14% of incidents of aspiration pneumonia and all rises in respiratory distress are 15% could be credited to emissions [84]. According to the putative virulence authorities, ozone, sulphur dioxide, nitrogen oxide and particulate matter are immoveable. Usually, these pollutants start from automobiles and the age of power, through the use of unsustainable energy production. Pollution is recommended to contribute to asthma through lipid peroxidation, which empowers flight course revamping; extended disruption, particularly through TH2 and TH17 pathways; and progress of aeroallergen [85]. Different tests have shown that allergy sufferers

introduced to pollution have lower lung functions, a more noticeable rescue drug care needed, and improved visit and hospital care gestures [86].

2.2.5 Risk Factors Related to Genetics

Although biological aspects have shown a tremendous impact in the improvement of the Asthma, gene mutations seem to respond. Researchers investigated the hereditary susceptibility of asthma (the frequency of change within the total over an overall population due to the maternal interaction of samples in that general population) have shown 35 to 95 percent evaluation [87]. 82 percent climate sensitivity was shown by a recent survey of more than twenty-five thousand Swedish twins which are 9 and 15 year in age [88]. While asthma is associated with many trace elements, asthma stimulation has similarly captured numerous characteristics. As expected, up to this stage, there are eight positionally implanted characteristics for asthma are: DPP10, ADAM33, PHF11, HLA-G, NPSR1, CYFIP2, OPN3 and IRAK3 [87]. In this connection, there are more than 100 member characteristics that are linked with asthma and unique figures found in genome-wide research studies (GWAS).Such features and positions are maintained in a public database of GWAS outcomes [89].

In recent analyses of these genetic risk factors [87]. A 17q21 locus and numerous mutations, furthermost notably ORMDL3 as well as GSDBB, are one of the most observed loci in asthma genomes [90]. This genotype was first identified by GWAS and has been continuously reproduced, particularly in childhood asthma [91]. This locus describes an advanced stage trait of asthma that modulates breathing problems and other pollutants [92].

2.2.6 Gender

Correspondingly, a few studies from all over the world suggest that women are at greater risk of asthma than men, since adolescent boys are at greater risk of asthma than teenage adults over the age of 14. By contrast, females are likely to have asthma across pre-adulthood [93]. The causes are hard to distinguish at this

stage: there is a difference in the occurrence of asthma at various ages. Certainly, women have wider lungs at birth than men, but in adults [94] , which may be one reason, they are smaller [95].

2.2.7 Obesity

Obesity appears to be well defined due to the apparent growth of irregular or unwanted fat, which can be harmful to health. It is said that 1 / 4 of the pediatric population is overweight [96] . While little is known about the biological roots of the relationship between diabetes and allergy, obesity is considered to be an important risk factor for disease care. In some of these countries, overweight people have a higher asthma rate than non-obese people and have a worse response to treatment, considering it a serious illness. A study of 1,001 monozygotic and 383 dizygotic fraternal twins found that the genetic component of obesity was correlated with 8% of asthma. He demonstrated that they are genetically related to both illnesses [97].

2.3 Managing Asthma

According to Global Asthma Initiative (GINA), effective symptom controls and the potential risk of death associated with asthma, fire, chronic airflow restrictions and the adverse effects of treatment are a long-term priority for asthma management. The use of pharmacological and non-pharmacological therapies includes asthma care. Treatments which are not pharmacological include the absence of smoking, physical activity and avoidance of causes and allergens in some patients, including, however, food allergies, mould, dust mites, and pollen. Asthma therapy is based on an exhaustive review including asthma control levels along with modifiable risk factors, co-morbidities, lung function and patient preference [98]. The degree to which asthmas manifestation is controlled, with or without treatment is known as asthma control [99, 100]. A pharmacological approach to asthma treatment is suggested by GINA (step-up if appropriate and step-down if possible) [98]. In practice, an increasing treatment is a typical reaction to uncontrolled asthma.

These recommendations recommend that health professionals should check on patient inhaling strategies and enforcement before contemplating any step-up of the procedure [101].

Most asthma patients require two forms of pharmacological treatment: quick relief (also called relief medicines) and control medicinal drugs (also considered to be 'longevity' or 'preventing medicines. 'Initially, asthma therapy relies on symptoms, lung functions and evolving risk factors while continuing care decisions rely on an individualized evaluation period, treatment change and reaction analysis [98]. Relief drugs with a short-acting beta2 inhaled agonist are used to treat breathlessness symptoms. Regular preventive drugs such as ICS and LABA are administered according to the nature of the symptoms and the required inhaler unit, as well as the combination of ICS and LABA (length-actingbeta2 agonist). These are used on a regular and long-term basis to reduce the risk of aggravation. In certain patients, ICS-LABA (formoterol for example) inhaler combination is used as a normal treatment for maintenance and as a relief drug for the patient. Additional therapy add-ons like long-acting muscarinic antagonists or biologists can be considered if patients are persistent and/or worsened given elevated ICS and LABA levels [102].

2.4 Treatment of Asthma

Treatment A cure has yet to be developed. Instead, clinical goals are focused on controlling the symptoms and preventing their intensification. Some of the major prescribed drugs are inhaled corticosteroids, beta-agonists with long-acting actions, leukotriene and theophylline. Treatment depends on the level of extreme condition, where mild sporadic cases do not need regular medication by the National Institutes of Health; mild persistent patients should use low inhaled corticosteroids; moderate persistent persistent individuals should use either small dose-inhaled corticoids with long-acting beta-agonists or medium-dose inhaled corticosteroids [5]. Inhaled corticosteroids are the primary treatment of asthma; however, they may be aided by additional medications. Inhaled corticosteroids alleviate

asthma symptoms by reducing the inflammation of airways. At a molecular level, they function within the nucleus, interacting directly with DNA to result in both an increased production of proteins that decrease inflammation and a decreased production of proteins that increase inflammation [103]. There are currently six types of inhaled corticosteroids on the market for clinical use. Their differences in pharmacokinetics allow for a variety of options to fit individual patients' needs. 12 Supplementing these inhaled corticosteroids with long-lasting beta-agonists can further alleviate asthma symptoms. Particularly, asthma attacks during the nighttime are highly correlated with hospitalization and mortality. Thus, another major clinical goal is to take preventative action against nighttime symptoms. While long-lasting beta-agonists are often prescribed for daily usage with inhaled corticosteroids (if used alone they can increase mortality), they are also highly effective in reducing nighttime symptoms [104]. In general, they are effective in reducing the risk of exacerbation of symptoms. They are, in turn, made more potent by inhaled corticosteroids which increase production of adrenergic receptors that present on the smooth muscles of the airways [103]. Another medication used to prevent nighttime symptoms is theophylline—a bronchodilator taken orally. This treatment's effectiveness is dependent upon the fact lung capacity contains high variability based on circadian rhythms [105]. However, this medication is prescribed less frequently as users exhibit more side effects compared to more common treatments such as long-lasting beta-agonists or corticosteroids inhaled. Another treatment that can be prescribed in conjunction with inhaled corticosteroids are leukotriene modifiers. Since many manifestations of asthma, such as increased mucus secretions and smooth muscle contraction, are the result of stimulation of the cysteinyl leukotriene (CysLT) 1 receptor, one treatment is preventing the stimulation of this receptor. Thus, CysLT antagonists can further 13 alleviate asthma symptoms. They are also shown to increase the effectiveness of inhaled glucocorticoids, allowing their dosage to be decreased [106].

The symptoms of breathlessness, cough, wheezing and decreased lung capacity steadily increase as asthma problems. Asthma in both children and adults is severely caused by viral lung infections. Any pathogen exists usually associated

with an increased risk of failure to treat [107]. In general, in the treatment of asthma disorders, beta-2-agonists receive more symptoms and increase use of orally taken glucocorticosteroids.

2.4.1 Glucocorticosteroids

The main agent commonly used in children with asthma which has good anti-inflammatory effects is the distant and far away glucocorticosteroids. In order to control all asthma conditions, beta-2-agonists symptomatically raise their doses, contributing to increased glucocorticosteroids use.

In current years, the increasing number of preclinical indications favor the use of long-term beta-agonists with drugs such as salmeterol and formoterol. The combined therapies for RV infection have been identified with in vitro salmeterol and fluticasone or together with multiple chemokines (CCL5, CXCL8, and CXCL10) and regenerative factors (FGF and VEGF) [108].

2.4.2 Omalizumab

Unfortunately, the incidence of contagious asthma was not noticeably decreased by systemic IGE care. Asthma conditions are treated with omeprazole until a year, most of them due to RV infections, to remove seasonal peaks [109]. The clinical benefit was the improved IFN-μ-response, in conjunction with IGE neutral drug, which could reinstall omalizumab in the antiviral response [110].

2.4.3 Anti-virus

Tests for his ability to concentrate on RV pollutants have been recent in laboratories and clinical research. These include soluble icam1, tramacamara and two antiviral drugs (pelicanic and rheumatoid arthritis) which interfere with the RV addiction in asthma patients following onset of viral respiratory infections, and IFN also inhalation. All these signs, however, have only a limited advantage in viral replication clinical symptoms and thus cold advances despite having major side effects.

Other treatments for asthma also introduce recently and work successfully for relief from this disease, these are discussed in (Table 2.1). currently approved pharmacological therapy for adult asthma. That is seems to be give perfect management of asthma.

TABLE 2.1: Pharmacological treatment approved for adult asthma [111]

Treatment	**Category**	**Treatment Step**
SABA	Symptom reliever inhaled bronchodilator	1 to 5
ICS	Preventative treatment against inflammation	2 to 5
Sodium cromoglicate or nedocromil sodium	Stabilizer inhaled in the mast	2 to 5
LABA	Symptom Manager Inhaled Bronchodilator	3 to 5
ICS/fast-onset LABA maintenance and reliever therapy	Additional doses for symptom relief Daily prevention of ICS/LABA therapy	3
Leukotriene receptor antagonist	Anti-inflammatory, soothing oral smooth muscles	3 to 5
Theophylline	Oral therapy of inflammation.	3 to 5
Bambuterol	B2 agonist for oral slow release	3 to 5
Tiotropium (via soft mist inhaler)	Bronchodilator of long-acting muscular antagonist	4 to 5
Omalizumab	Monoclonal anti-IGE antibiotic	4 to 5

Maintenance oral corticosteroids	Systemic therapy against inflammation	5

2.5 Comorbidities and Asthma

It is possible that persons complaining of symptoms of perfume or of other sensory irritants would not be able to have an IgE-mediated reaction, which is the cause of an allergic reaction [112]. The cause of intolerant symptoms has been suggested that they are differentiated by asthma/allergy, although some suggest that exposition to toxic substances can lead to occupational asthma and multiple chemical sensitivities (MCS) [112]. Symptoms of EI are typically associated with substances that produce odor and sensual discomfort in the general population (e.g., perfumed products) and with bio-associates (e.g., mold) (e.g., pungency). These signs are usually regarded as unexplained medically [113] Since etiology is unknown and because successful medical treatment is not understood. Asthma/allergy, for instance, airways, mucosae and general symptoms (e.g. fatigue) are normal in MCS and in buildings with symptoms associated with non-specific buildings [114]. In both allergies and self-identified chemical (smell) aversion Meggs and Dunn (1996) reported fluid/stuffy nose, eye irritation, sinus symptoms and shortage of the breath to common. Since asthma, allergy and intolerance are similar in nature, an increased risk of overdiagnosis or misdiagnosis can occur in actual cases of EI which might be followed by mismedication without major impact. This may result in an increased risk of over-diagnosis or allergy. Chemical sensitivity (CI) is an EI that is symptomatically due to low concentrations of (typically fragrant) chemical items such as perfume, cleaning products and flowers. CI is generally known as self-reporting (i.e., positive responses to questions such as 'do you have odorous exposure symptoms') or provided by a doctor. MCS is the most common clinical definition, medical diagnosis, but even labelling, including idiopathic intolerance in the environment and sensory hyperreactivity, is used [112]. The prevalence of self-reported CI is remarkably high and ranges between 9% and 33%

[115–117], and satisfy CI diagnosed between 0.5 and 6.3% criteria [112, 118]. The other kind of IT is a form which is referred to as building aversion (BI), or NBRS if diagnosed by the doctor (also called "sick building syndrome"). The other approach is to assign symptoms in such buildings. Similar to CI, BI is sometimes listed as self-reported or diagnosed as a physician. BI has a prevalence of 4.3 to 5.0 percent, and self-reported and physician-diagnosed intolerance of 2.5 percent respectively [119, 120]. CI and BI are also considered as closely related disorders or for this reason other EIs as sound hypersensitivity and the symptoms due to electromagnetic fields. They are identified as symptoms that are medically explicit and perhaps underlying. A broad co-and multimorbidity also exists among different EIs [121]. A Japanese study found that 84% of patients with MCS had a pollen, house dust or medicinal products allergy [122]. Comorbid aversion such as CI and BI, can increase stress, social isolation and decrease in QoL in asthma and allergy. Such a viewpoint might help to explain potential factors that may have a role to play in asthma and allergy and help to enhance understanding of PNI processes.

In recent cluster trials, asthma has been shown to be a heterogeneous condition that displays distinct phenotypes rather than a single disorder [123–125]. Age at the onset of asthma has become a crucial factor for the detection of phenotypes. Adult or late-coming patients with asthma are typically un-atopic, have a lower prognostics and are more likely to have recurrent airflow limitations [124, 126, 127]. In infancy most asthma should start. Nevertheless, adult asthma has been recently called into question in women over the age of 40 in the US [128]. Asthma and adult phenotypes were associated with factors such as women's sex, obesity, proximity to work, rhinitis, respiratory infections, smoking, stressful life and poor lung function.

ABPA: allergic bronchopulmonary aspergillosis; COPD: chronic obstructive pulmonary disease; GERD: gastroesophageal reflux disease; OSA: obstructive sleep apnea [129, 130]. In patients that are otherwise healthy, most clinical trials with asthma have been performed. Comorbid conditions are very common as shown in Fig 2.1, however, in real life, particularly in patient's adult and/or older. In 35% of adults, for example, are obese [131].

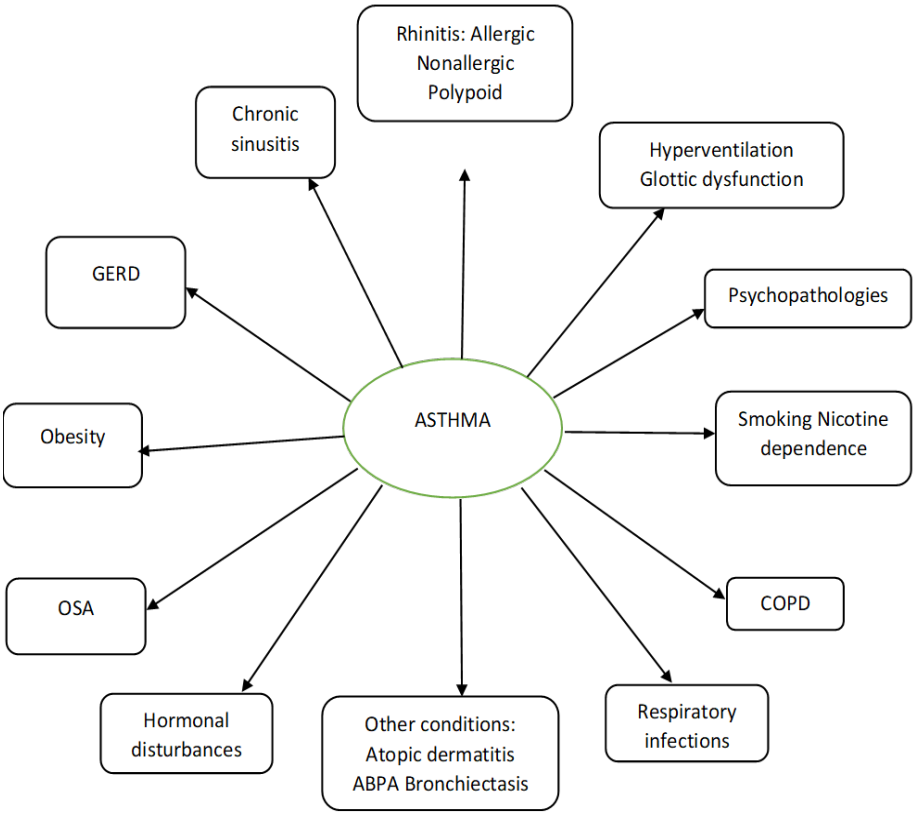


FIGURE 2.1: Some common asthma-related comorbidities

2.5.1 Epidemiologic Studies on Comorbidities in Asthma

A complete asthma drug prescriptions included a total of 37060 patients in a sample of 1239533 subjects aged 8 to 29 years [132]. This study found an increased than predicted 59 percent of asthma patients had one or more of those chronic diseases in all investigative conditions, including This study found an increased than predicted 59 percent of asthma patients had one or more of those chronic diseases in all investigative conditions, including (migraine, psychiatric illness, ADHD, epileptic disease, cardiovascular, autoimmune, gastroesophageal reflux diseases, allergic, antibacterial use and anti-viral disease) except diabetes, with a general population 18 percent higher than expected with a general population 18 percent higher than expected. The highest odds for other asthmatic diseases in 20–29-year-olds were allergies (male codes were4,8,4,1), GERD (male codes3,2,3,5) and ADHD; (odd ratio in men 2.3 ,2.5in women). The population of the sample aged between 8 and 29 years showed a high concomitance prevalence [133].

2.5.2 Sinusitis & Rhinosinusitis

Asthma incidence is unrelated to 75% of asthma patients with chronic rhinosinusitis [134], In up to 84 per cent of severe asthma patients, while sinusitis evidence can be measured by sinus computed tomography (CT). On the other hand, in a population of around 4-10 percent of asthma self-reported (determined through written asthma attacks in the previous year or presently using asthma medication), the prevalence of asthma in CRS patients may fluctuate between 11 and 42 percent depending on the diagnostic mean [135, 136].

2.5.3 Gastroesophageal Reflux Disease

The risk of GERD-related symptoms is also significantly higher among Asthmatic patients than the population overall. The risk of simultaneous asthma in GERD patients is suggestively greater compared to patients without GERD [137]. The prevalence of abnormally esophageal PH in patients is, however, highly variable from 12 to 85%, compared to 50 to 80% for asthmatic patients with GERD symptoms. In addition, large portions of the latter have quiet GERD and have no definitive heartburn symptoms. In addition, large portions of the latter have quiet GERD and have no definitive heartburn symptoms. Asthma can be aggravated by direct effects on airway sensitivity or aspiration-inducing inflammation, due to gastroesophageal reflux disease. On the other hand, asthma bronchoconstriction and asthma can lead to gastroesophageal reflux. This indicates that asthma and asthma medications can increase GERD and that GERD can contribute to asthma symptoms. GERD is the result [138, 139]

2.5.4 Asthma and the Corona Virus Disease

A special note in the United Kingdom is the association between asthma and a greater risk of death from Covid-19 in hospital. In some countries where asthma and other chronic respiratory problems were underestimated in hospitalized patients, this discrepancy is close to previous findings [140]. In the UK, 14.0% and 17.9% of hospitalized patients have also reported asthma but no increased risk of

death [141]. Viral infection apparently accounts for up to 85% of asthma exacerbation in school children and viruses are isolated from symptomatic patients more often than from asymptomatic patients [142]. Even then respiratory viruses are one of the most common causes of asthma intensification in any age group, not all of which correspondingly affect patients. Some people defined human rhinovirus as the key contributor to asthma exacerbations, with the presence of coronavirus one of the less well known causes for asthma exacerbations in children and adults [143, 144].

2.5.5 Allergic Rhinitis

A persistent inflammation of the nasal airways in response to allergens is allergic rhinitis [145] where an IgE-mediated response triggers the reaction. Nasal inflammation, itching, rhinorrhea and sneezing are typical signs of rhinitis. Rhinitis nasal symptoms can also include ear, eyes and throat symptoms such as runny and itchy eyes and palate or pharynx itching. Rhinitis is one of the most common diseases in the world and affects up to 40% of some populations [146]. Rhinitis is one of the most common diseases in the world and affects up to 40% of some populations [147]. Extreme rhinitis has been found to cause not only nasal symptoms but also decreased QoL and sleep performance and work performance [148]. It is estimated that about 14-15% of the Swedish population is affected. Atopic dermatitis A chronic inflammatory skin disease, characterized by severe itching and skin lesions, is known as atopic eczema Atopic dermatitis. Atopic dermatitis is commonly documented first during infancy and later in adult or adult life [149]. In early infancy, infants are most affected by scalp, forehead, neck and trunk, while the bending surfaces of extremities like the fold/back of the knee and back of the elbows, along with the neck, wrist and angle, are more normal in older children. The bending surfaces of the limbs, hands and feet are also the affected areas of the body in adolescent and adult life. Popular for all ages is that itching is present all day and worsens at night, leading to sleep loss and decreased QoL. Like asthma, atopic dermatitis is allergic or non-allergic. In some nations, 10-20% of kids but 1-3% of adults are thought to have been affected [150]. In a recent

study the prevalence of atopic dermatitis in Sweden was indicated to be up to 15% [151]. Comorbidity within asthma and allergy A risk factor for other atopic diseases such as allergic asthma and rhinitis has been proposed previously. In an Akdis and colleagues analysis [152]. It had been recorded that in the next two years 50 percent of children who developed atopic dermatitis developed asthma. Those who later developed asthma or allergic rhinitis were also recommended to develop a more serious disease. Similar atopic diagnoses, including asthma and allergic rhinitis, have been related to, and showed in the strong epidemiological, pathogenic and immunological combinations, by others Asthma and rhinitis [153]. In previous studies it has been debated if these two atopies should be divided into various diseases or if one should consider them to be the same, while different sites are present. This relationship is defined as the hypothesis of integrated airways with allergic respiratory diseases. Asthma and rhinitis are therefore known to be symptoms of a continuous respiratory process rather than independent illnesses [154].

2.6 Pathways Involved in Asthma

2.6.1 Signal Pathway IL-4 and IL-13

This JAK/STAT pathway is triggered by allergic cytokine receptors, including IL-31, IL-4, IL-13, IL-5 and thymic stromal lymphopoietin [155]. This seems to be the most common route involved in Asthma etiology. Two separate heterodimeric IL-4 receptors, type I IL-4R (comprising il4rp and common cytokine receptor chains) as well as type II IL4R (comprising IL-4RP and IL-13RP1 chains) are recruited by the signaling pathways stimulated by IL-4 and IL-13Though some IL-4 attached to the receptors IL-13 of type I interferes with the receptor of type II called IL-4RS. By manipulating all forms of IL-4 receptors, in the stimulation of STAT-6 transcription factors phosphorylation of JAK1, JAK2, and TYK2takes place in their results and occurs in organic phenomenon of target immune cells[156] Consequently, the IL-4/IL-13 axis suppression provides a beautiful Asthma physiological role. A central modulator of Asthma etiology could be the IL-4, IL-13 and STAT-6 route.

The STAT-6 initiation is often blocked by overlap with ERK1/2 as well as P38 interactions with STAT-6-MAP, and the suppressing serine stat-6 phosphorylation, the prevention of STAT-6 acetylation, and the suppression of P300 initialization gene expression coactivator [157]. The serine kinases ERK, P38 MAPK, JNK, and MTOR transactivate STAT-6 by phosphorylation of its proline residues [158].

2.6.2 Adiponectin Signaling Pathway

Obesity has been linked to increased inflammation of the airway, AHR (Aryl hydrocarbon receptor, oxidative stress, transmission of inducible gas synthase (INOS) and excessive gas (NO) levels hazardous used for Asthma. Obesity, however, is characterized from a reduced amount of adipokine, that works to minimize the occurrence of allergic asthma as either an inflammatory and anti - oxidative facilitator [159]. Adiponectin stimulates receptor adiponectin 1 (ADIPOR1) and recipients of adiponectin 2 (ADYPOR2), T-cadherin and calreticulin [160]. Adiponectin forms complexes with ADIPOR1 and several other receptors, by activating AMP-activated protein kinase (AMPK) and peroxisome-activated proliferator-alpha receptors. AMPK(AMP activated protein kinase) controls cellular metabolism (and obesity) as an essential energy indicator, often due to the inflammatory functions of macrophages [161]. A critical infectious signaling pathway may be part of the nuclear factor kappa-B (NF-EARB) [162]. The NF-3B family consists of 5 members from RelA (p65), RelB, c-Rel, p50/p105, p52/p100 and mammalian cells (NF-3B2) [163]. These studies discovered that in obesity-related asthma, the frequency of adiponectin substantially decreased. It was also suggested to prevent inflammation of the airways and oxidative stress in obesity-related asthma. The most common sources of myeloperoxidase are neutrophils, while eosinophils primarily contain exotoxin. It was also suggested to prevent inflammation of the airways and oxidative stress in obesity-related asthma. The most common sources of myeloperoxidase are neutrophils, while eosinophils primarily contain exotoxin. The level of MPO (Myeloperoxidase) was higher in obesity than severe asthma, suggesting that neutrophil and eosinophil infiltration respectively were the main pathogens in these syndromes [164].

2.6.3 Signaling Pathway of the Prostaglandin D2-(PGD2) Receptor

A pro inflammatory facilitator derived by arachidonic acid in the cyclooxygenase-2 (COX-2) pathway can be PGD2(Prostaglandin D2). PGD2 is released mainly from the mast during inflammatory reactions from an activated immune system [165]. PGD2 can also be activated at very low (μmol) concentrations with two receptors: PGD2 receptors 1 and a few (DP1 and DP2)21. Dp2 may be a G-protein-coupled receptor expressed as homologous chemical attractant receptor molecule expressed on the membrane surface of TH2 cells, mast cells, and eosinophils (CRTH2) [166]. Binding PGD2 to the DP2 receiver causes proinflammatory signals which eventually activate and include TH2 cells and eosinophils in inflammatory areas of asthma [167].

2.6.4 Signal Pathway of NF-KB-inOS-COX-2

After phosphorylation and disorientation, the NF-KB may become an all-round transmembrane protein activate by IKB kinase its sub-unit inhibitor kapa-B alpha. The NF-KB-induced INOS and COX-2 are important mediators in pulmonary inflammation development. In addition, the activated NF-KB controls the INOS and COX2 levels [168]. By comparison, NF-KB enables INOS and COX-2, which could lead to other inflammatory mediators and cells [169]. INOS and COX-2 changes are therefore essential in the control of lung and airway inflammation. INOS and COX-2 changes are therefore essential in the control of lung and airway inflammation.

2.6.5 Interferon-virus Pathway

Class I (IFN-alpha and IFN-β) interferons play an important role in antiviral inflammatory responses. Pattern Receptor Recognition Social Immunity (PRRS), including a toll-like receptor 3 (TLR3), I-protein and melanoma-associated [170]. In 2018 there are low transcriptions of IFN-alpha and IFN–β in patients with a psoriasis within the epithelium and sub-epithelium macrophages. IFN-alpha and

β defects in airway epithelium macrophages, as well as sub-epithelium deficiencies linked to severity of a viruses infections and asthma, such as rhinovirus, glycemia, etc [171].

2.6.6 WNT/β-catenin Signaling Pathway

In mammals, 19 members of the WNT family have a crucial role to play in regulating biological processes. The WNT signals pathway is divided into canonical (β-catenin addiction) and non-canonical pathways (β-Catenin addiction)[68]. In asthmatic patients, dysregulated Gene expression has been related to the pathogenesis of airway renovations. As a consequence, WNT/β-catenin is involved in intracellular accumulation and nuclear transfer in the lung maturity, thereby contributing to endothelial airway component formation. The activation of the WNT signaling pathways has also been shown to increase the multiplication of airway smooth muscle cells involved in airway remodeling [172]. Asthma also correlated to the family gene encoding with a (fam13A) cross-related sequence 13A. FAM13A, interestingly, regulates β-catenin stability in asthma and enhances WNT signaling. Polymorphisms in two genes, the WNT-1-protein inducing pathway-1, and the WNT neurotransmitter factor-1, are ultimately associated with chronic asthma [173]. Vitamin D is involved in manipulating innate and adaptive immune reactions. Deficiency in vitamin D improves asthma incidence and reduces glucocorticoid tolerance [174]. The bio-active vitamin D type (1,25(OH) 2D3) also stimulates the translocation of β-catenin to the cell wall from the nucleus, removes β-catenin-transcriptional TCF-4's functions and eventually activates the DCKOPF-1 gene transcription, coding WNT extracellular inhibitor [175]. β-Catenin is important for the permeability of the cytoskeleton. Vitamin D also decreases expression of WNT5A and Bencatenin and substantially inhibits the role of the WNT and β catenin signaling pathway which reduces asthma remodeling of the airways. Moreover, 1,25(OH) 2D3 also increases the proliferation of smooth airway muscle cells and decreases its alpha-SMA content. Higher levels of alpha-SMA are thus features of aerial restructuring besides an increased airway wall thickness and collagen deposition [176].

2.6.7 Pathway for FOXC1-MIR Signals

The short non-coding RNAS Micro-RNAS (miRNAS) involved in post-transcriptional gene regulation (∼22 nucleotides long). The 3'-untranslated regions of MRNAS are attacked by MIRNAS, causing their degradation and finally inhibiting their translation [177]. The 3-kinase (PI3K)/AKT signaling pathway in allergic asthma plays a regulating role and is monitored mainly by MIRNAS. In hypoxic pulm, the bifurcated head BOX C1 (FOXC1) is able to inhibit a hypoxia factor of the fox transcription factor [125]. The transcription factor BOX C1 (FOXC1), a factor caused by hypoxia in the family of fox transcription factor, is capable of inhibiting the hypoxic pulmonary lungs. Blocking endothelial-mesenchymal transformation by blocking TGF-β1 by reducing FOXC1 expression also decreases fibrosis of lung-tissue. Blocking endothelial-mesenchymal transformation by blocking TGF-β1 by reducing FOXC1 expression also decreases fibrosis of lung-tissue. FOXC1, which results in phosphorylation and active of several corresponding proteins such as NF-KB and GSK3-B is activated for the PI3K/AKT signaling process [178].

2.6.8 JNK-GAL-7 Signaling Pathway

In cellular apoptosis, TGF-β1, was also used as mediator in peribranchial fibrosis, and in asthma airway remodeling. Galectin-7 (gal-7) may belong to the family of galectin. This molecule is expressed on epithelial cells and interacts with β-Galactosidase. The p53 activates the Gal-7 gene and has pro-apoptotic implications. In bronchial asthma epithelium, high expression of Gal-7 was noted [179]. Silencing in airway epithelial cells was shown to inholed TGF-β1-induced apoptosis. Caspase 3 has been associated with the inhibiting effect of the GAL-7 on TGF-β1-mediated apoptosis and thus BAX, BCL-2 and PARP are induced. Gal-7 can be a mitochondrial bonding and disabling BCL-2 partner. Caspase-3 and its downstream substratum PARP, however, initiate early apoptotic events. PARP Cleavage could be a significant marker of activation for functional caspases in bronchial epithelial asthma cells and an indication for apoptosis [180]. Studies found that the siRNA of Gal-7 decreases the activity of caspase-3, cleavage and

BAX expression while increasing the expression of Bcl-2 [181]. The JNK signal pathway is also influenced by TGF-β. In the sense of apoptotic process and asthma airway remodeling through activation of the signal's pathways of Wnt5a/JNK, a JNK, a stress-actively kinase protein, which is a member of the MAPK family, is important. TGF-β1 activates serine residues 63 and 73 JNK, phosphorylates Jun's substrate [182]. Conversely, silencing Gal-7 prevents JNK activation and enhances bronchial somatic cell injury and is a potential target of asthma treatment [183].

2.6.9 NRF2-ROS Signaling Pathway

Airway inflammation and asthma are associated with reactive oxygen (ROS) bacteria. In airways, the primary origins of ROS are epithelial cells and neutrophils. The nuclear erythroside factor 2-related factor (NRF2) is transcription by activating downstream proteins including NADPH quinone oxidoreductase (NQO1) and heme oxygenase as key oxidative stress regulator and as a pulmonary fibrosis (HO-1). Chronic inflammation also increases the expression of NRF2induced TGF-β, which also has a central role in the development of lung fibrosis. Therefore, eliminating the upstream signals leading to ROS development has a possible therapeutic effect on asthma [184].

2.6.10 FOXP3-RORγT Signaling Pathway

In the peripheral blood of the asthmatic patients the proportion of CD4+CD25+ TREG cells is decreased. Some research showed that the TREG/TH17 imbalance contributed to asthma severity [185]. FOXP3 may constitute a key transcript factor for the regulation and development of TREG functions. The equilibrium between foxp3 and RORγT therefore regulates the ratio TREG/TH17. RNAS (LNCRNAS) for a long duration of noncoding include ∼200-nucleotide-long RNAS involved in airway inflammation and asthma pathogenesis. LNCRNAS take part in posttranscriptional regulations of various target and protein genes [186]. LNCRNAS may be an endogenous competing RNAS (CERNAS), binding them to the microRNA and depriving them of binding on the target MRNAS. LNCRNAS (i.e., CERNAS) in asthma influence the amount of Foxp3 and ROR NATO indirectly, by

targeting their particular miRNAs, thus leading to the imbalance of TREG/T17, a function of asthma pathogenesis. Though the TREG/TH17 balance is regulable by LNCRNAS, it remains important to explore other possible mechanisms. Finally, MIRNAS and LNCRNAS are potential regulators of asthma immunological responses and may be used in the treatment and diagnosis of the disease [187].

2.6.11 MAPK-NF-KB Signaling Pathway

Asthma inflammation is controlled via the NF-KB and MAPK signaling pathways by controling the organic phenomenon of inflammation factors such as TNF-α and IL-6. In 2019, it demonstrated the ability of asthma inflammation mediators to regulate the IGE and il-4 assembly by nuclear translocation of the phosphorylated p65, IKB inhibition within the NF-KB signage pathways, and ERK, JNK, and p38 MAPK phosphorylation (i.e. activation of MAPK signaling pathway) [188].

2.6.12 CYSLTR Signaling Pathways

There has been some evidence that Cysteinyl leukotrienes and their receptors contribute to allergic asthma. Two kinds of CYSLT receptors, CYSLTR1 and CYSLTR2, are in the g-protein-coupled receptor family. It is reported that CYSLT C4, D4 and E4 modulate inflammation of the airways and restructuring [189]. Two kinds of CYSLT receptors, CYSLTR1 and CYSLTR2, are in the g-protein-coupled receptor family. Two kinds of CYSLT receptors, CYSLTR1 and CYSLTR2, are in the g-protein-coupled receptor family. CYSLT E4 is the powerful mediator for evoking the inflow of eosinophils, basophiles and enhanced AHR and mucus secretion, despite its low affinity for CYsLTR1 and a number of them. While montelukast and pranlukast are two CYSLTR1 antagonists and several, there are no known CYSLT E4 antagonists. The GPR1 or GPR99 2-oxoglutarates may be a new CYSLT E 4 receptor and activation enhances the CYSLTR1/CYSLTR 2 pathway vascular permeability. P2Y12R is also an eosinophilic degranulation and inflammation modulator induced by CYSLT E4. CYSLT E4-induced degranulation and inflammation by P2Y12R antagonists suppress asthma and perhaps new candidates for inflammation and bronchoconstriction in this condition [190].

2.6.13 CAMP Signaling Pathways

Two of the main inflammatory regulators are cyclic 3′5′-adenosine monophosphorus (CAMP) and cyclic guanosine monophosphate (CGMP). Following their phosphodiesterase (PDE) hydrolysis, intracellular depletion of both CAMP and CGMP increases inflammatory response. The removal of the PDE4, a subtype of leukocytic PDE enzyme, has encouraged anti-inflammatory effects in asthma. The removal of the PDE4, a subtype of leukocytic PDE enzyme, has encouraged anti-inflammatory effects in asthma [194]. CAMP is also a negative T-cell activation regulator. PDE4 inhibitors have thus suppressed T-cell cytokine production, as well as type 2 inflammation biomarkers like periostin and serpinB2 in asthma. A possible therapeutic role in allergic asthma may also be the control of TH2-mediated reactions e.g., IL-4, IL-5, and IL-13 assemblies (e.g., IL-4, IL-5, and IL-13 assemblies). The CD28 stimulating receptor activation induces PDE4 leading to the camp hydrolysis, NF-KB, protein-1 activator (AP-1), NFAT, and also because T-cells are activated and proliferated [191].

2.6.14 FAS-FASL Signaling Pathways

FAS can be a member in an activation-induced necrobiosis family of TNF receptors. Asthma is flawed due to the FAS-mediated signaling and delayed inflammatory resolution. The expression of FASL has been shown to be increased following allergens exposure. The FAS on the surface, however, has been less susceptible to asthma FAS-mediated apoptosis in pulmonary T-cells. The FAS on the surface, however, has been less susceptible to asthma FAS-mediated apoptosis in pulmonary T-cells. In addition, the number of cells expressing the BCL-2 antiapoptotic molecule in asthmatic patients and asthma-related severity has been increased. FAS was also mentioned to control inflammation caused by TH2 [192]. Two apoptotic and nonapoptotic signaling waterfalls are initiated. FAS ligation modifies its composite structure, allowing signaling molecules i.e. FADD, CFLIP and procaspase-8 (i.e. FADD, CFLIP and procaspase-8) to bind to the receptor's intra-cellular c-terminal signaling death domain [193]. FAS can be a member in an

activation-induced necrobiosis family of TNF receptors. The expression of FASL has been shown to be increased following allergens exposure.

2.6.15 PTHRP / PPARγ Signaling Pathway

The alveolar type II (ATII) cells in the physiological state are secreted by parathyroid hormone-related protein (PTHRP) and prostaglandin E2. The alveolar type II (ATII) cells in the physiological state are secreted by parathyroid hormone-related protein (PTHRP) and prostaglandin E2. The alveolar type II (ATII) cells in the physiological state are secreted by parathyroid hormone-related protein (PTHRP) and prostaglandin E2. Peroxisome gamma-activated receptor proliferator (PPAR) (also known as glitazone or nuclear receptor subfamily1, group C, member 3-NR1C3-) can be nuclear receptor type II. In nicotine induced pulmonary dysplasia, the PTHRP/PPARγ penetration signaling pathway was reported to occur in offspring. The bonding of PPARγ acid to PTHRP causes the processing by absorbing neutral lipids of lung fibroblasts into lipo-fibroblasts. It also updates PPARγ encounters by activating protein kinase an a (PKA). PPARγ also encourages adipocyte-related protein downstream and induces lipo-fibroblasts and ATII cells to consume triglycerides and secrete leptin. A surfactant is produced to guarantee normal lung function after it is attached to ATII cells. It may be possible for PPARβ agonists to support normal lungs and to inhibit dyspnea, to modulate the pathway of the PTHrP-PPARγ which leads to pulmonary dysfunction, especially for allergic asthma [194, 195].

2.6.16 PAI-1 Signaling Pathway

Asthma severity and airway remodeling were correlated with the Urokinase inhibitor-1 (PAI-1). Plasminogen is converted to plasmin by Tissue-type urokinase (TPA) or urokinase-type PA (U-PA). Plasminogen is converted to plasmin by Tissue-type urokinase (TPA). Plasminogen activators are involved within the dissolution of fibrin polymers and therefore the degradation of extracellular matrix (ECM) components. PAI-1 can inhibit both T-PA and U-PA. PAI-1 deficiency prevents ECM deposition and reduces airway inflammation and remodeling, also as AHR

(aryl hydrocarbon receptor). Therefore, that specialize in PAI-1 antagonists is often a viable therapeutic strategy in Asthma [196]

2.6.17 FCϵRI Signaling Pathway

Basophils express IGE receptors (FCϵRI) of high-affinity on their cell wall. FCϵRI activation results in chemical mediators such as histamine being discharged. By developing TSLP and IL-4 in response to protease allergens, Basophils guide the differentiation of naive T cells to TH2 cells in lymph nodes. By activating memory B and T cells, basophils also enhance humoral memory responses [197]. By releasing histamine and other mediators after activation by IGE-allergen complexes binding to FCϵRI on these cells, mast cells also play a crucial role in allergy. Tyrosine kinases such as LYN, FYN, and SYK are activated by the attachment of IGE-allergen immune complexes to FCERI, which subsequently phosphorylates the distribution of signaling molecules such as LAT, SLP-76, and PLC-γ1 and induces mastocyte degranulation. Highly active mediators, including histamine, prostaglandins, leukotrienes, heparin, serotonin, inflammatory cytokines (such as IL-6, TNF-alpha, MCP-1, etc.) and neutral proteases, are present in the granules of mast cells. Highly active mediators, including histamine, prostaglandins, leukotrienes, heparin, serotonin, inflammatory cytokines (such as IL-6, TNF-alpha, MCP-1, etc.) and neutral proteases, are present in the granules of mast cells. Generally speaking, pathways involved in mast cell activation are possible targets for the production of successful medicines to regulate allergic asthma attacks [198].

2.6.18 TIM-3-GAL-9 Signaling Pathway

Macrophages distinguish between two subtypes during inflammation: M1 (classically activated) and M2 (i.e., alternatively activated). CD86 expresses macrophage M1, secrete and activate INOS to inflammatory responses proinflammatory cytokines. On the contrary, macrophages of the M2 express CD206 and require immune tolerance and control. M2, also as Arginase-1, facilitate tissue repair and release anti-inflammatory cytokines [199]. T-cell immunoglobulin mucin 3 is a strongly expressed immunomodulatory receptor for TH1 and cytotoxic T cells.

Tim-3 induces THL and cytotoxic T cell apoptosis and controls NK cells, NKT cells and macrophages activity. Galectin-9 (GAL-9), when bound directly to tim-3 on TH1 cells, can be a TIM-3-driving ligand and peripheral immune tolerance. TIM-3/GAL-9 inhibits the function of macrophages and decreases inflammatory factors discharge. The high expression p-IŢB and p-P38 levels in eosinophils translate macrophagic polarization from M1 to M2 and lower inflammation by reducing both TNF-α, IL-6 and IL-12, as well as the CD68-positive macrophages. Eosinophils may also cause inflammation of type2, which is the main disease process in allergic asthma, on the other hand [200]

2.6.19 TLR Signaling Pathways

Antigen cells, particularly dendritic cells (DCS), identify allergens in atopic individuals. These cells present antigens to naive CD4t cells and cause differentiation in TH2 cells following their migration to lymph nodes. The TH2 immune response is consistent with pathogenesis and allergic asthma progression. The TLRS and NF-KB are essential functions in this method. TLRS identifies antigens by molecular patterns (PAMPS) associated with pathogens or molecular patterns associated with damages (DAMPS). Trigger NFKB (via IKK α/IKKB), AP-1 (via MAPKS) and IRF 3 signaling pathways (via TBK1, IKKE, and IKKA). Asthma is associated with genes and mutations of TLR signaling pathways such as nod1, NOD2, IL1RL1, MAP3K7IP1, and BPI [201]

2.6.20 PAR2 Signaling Pathways

Asthma bronchodilation is involved in protease-activated receptor 2 (PAR2). It has been studied as the therapeutic target of the asthma. PAR2 is nearly like β2-AR, which activates G-protein-dependent pathways for intracellular signaling. Thus, it can be therapeutic to asthma if unique ligands concentrate on this pathway. B-arrestins are GPCRS-recruited adaptor proteins for desensitization and internalization of consumer receptors. These proteins can also activate g-protein-independent signals by extracting the GPCRS from the cognate heterotrimeric Gα sub-units and reducing their reaction to agonistic stimulation [202].

2.6.21 KEAP1/NRF2/are Signaling Pathways

Signaling pathways of the NF-KB, MAPK, and JAK-STAT are involved in inflammation development (signal transducer and transcription activator). On the contrary, the transcription factor (NRF2) regulates expression of NADPH, NAD(P)h quinone oxidosis1, peroxidase, ferritin, heme oxidase-1 (ho-1) and other detoxification enzymes, which are related to anti-inflammatory and antioxidant factor2 [203]. NRF2 transcription factor controls. NRF2 belongs to the subfamily Cap 'n' collar (CNC) and includes 7 functional fields: (NRF2-ECH homology). NEH1, as a CNC-BZIP domain, allows NRF2 to heterodimer with the tiny MAF protein and develop a UBCM2 ubiquitin combinations complex Enzyme. The effectiveness of Nrf2, which should be revealed in future studies, in the treatment of asthma [204].

2.6.22 Ca2+ Signaling Pathways

Airways smooth Spasm modulates GPCR agonists and calcium pathways (Ca2+), which are dependent and independent. Phospholipases B generate an inositol triphosphate (IP3) that binds to the sarcoplasmic reticulum (SR) IP3 receiver and induces Ca2+ release into cytosol within the Ca2+ dependent pathway. Then, the intracellular Ca2+ induces phosphorylate myosin light chain kinase in calmodulins and myosin and activates cross-bridges of actin-myosin, caused by smooth spasm. The expression CD38 in parallel with this pathway evolves the production of cyclic ADP-ribose, which connects with the ryanodine receiver and promotes the release of Ca2+I from SR. On the other hand, the cytosolic Ca2+-ATPASE sarcos/endoplasmic reticulum refills SR with Ca2+I. Ca2+I release effects were elicited in allergies, methacholine, histamine, thrombin and leukotriene D4 [205]. During allergic asthma assaults, IL-13 is overexpressed, increasing the mobility of canonical calcium, enhancing calcium sensitivity and worsening asthma. RhoA removal was also reported for relaxing airway smooth muscles. These modulators can be useful for asthma symptoms and treatment [203]

Chapter 3

Methodology

3.1 Block Diagram

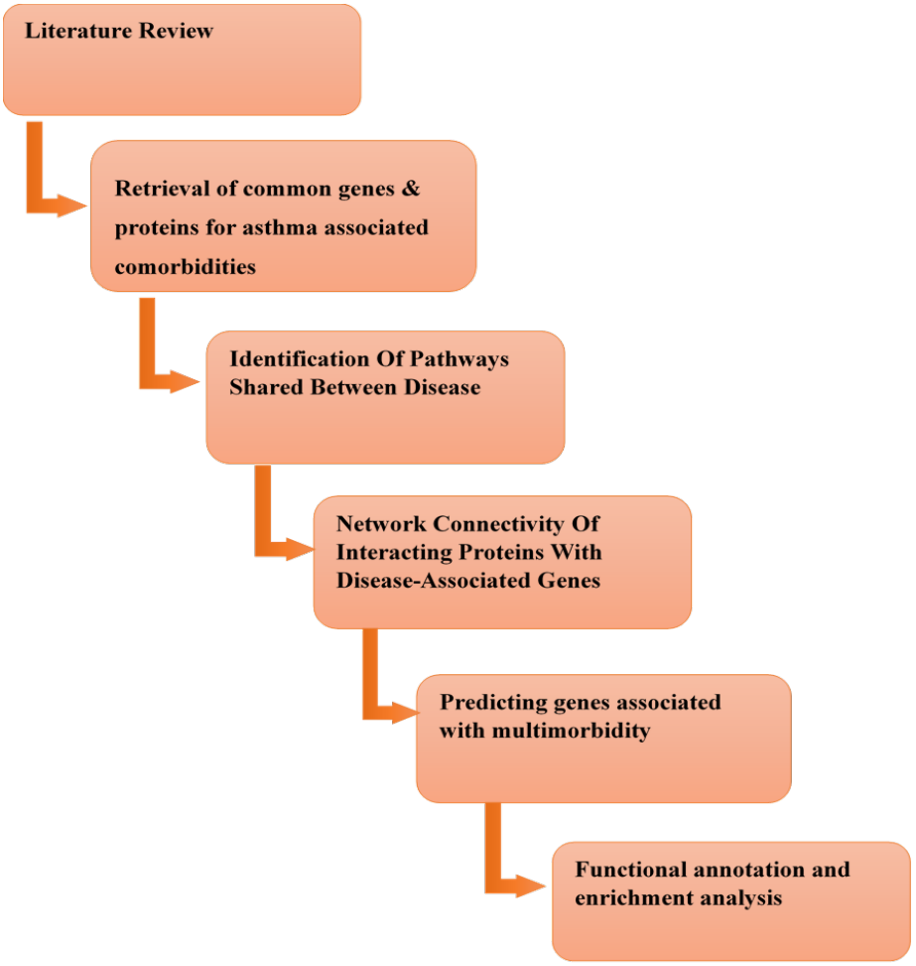


FIGURE 3.1: Flow Chart of Methodology Conducted for the Research

In silico-based research was conducted to identify the association of Asthma with Sinusitis, Gastroesophageal reflux and Rhinosinusitis. In this study common genes and proteins were retrieved. In order to determine allergic and non-allergic multimorbidity pathways, a functional network of associations was also created. Finally, a network-based approach provided a list of newly predicted multimorbidity related proteins.

3.2 Selection of Diseases

Asthma may be correlated and affect its clinical expression with many comorbidities, although its specific effect remains categorized. Asthma may be correlated and affect its clinical expression with many comorbidities, although its specific effect remains categorized. The impacts and treatment responses of co-morbidities on the pathophysiology of asthma are of great concern. Allergic diseases have many associations between them and also their management identified but the association with Gastroesophageal reflux disease with other diseases is not well defined. Diseases were selected Rhinosinusitis, Sinusitis, Gastroesophageal Reflux disease and Asthma were selected as comorbidities.

Asthma is co-morbidly chosen for rhinosinusitis and sinusitis, as 75% of asthma patients have persistent symptoms of rhinosinusitis depending on the severity of asthma [134].

Asthma is co-morbidly chosen for rhinosinusitis and sinusitis, as 75% of asthma patients have persistent symptoms of rhinosinusitis depending on the severity of asthma. Patients with asthma have a greater risk of symptoms linked to GERD than the overall population. GERD patients have a significantly greater chance of overall asthma than GERD patients do. GERD patients have a significantly greater chance of overall asthma than GERD patients do. GERD patients have a significantly greater chance of overall asthma than GERD patients do. However, irregular esophageal PH dilation ranges greatly from 12-85 in patients with asthma, while in 50-80 percent of patients with asthma, GERD symptoms are observed.

Furthermore, the last one percent have silent GERD and do not suffer typical side effects such as nausea [138, 139].

3.3 Retrieval of Common Genes and Proteins for Asthma Associated Comorbidities

Involvement of specific genes in associated diseases, as well as the genes associated with the specific proteins so there the whole work depend upon the identification of their interaction with each other and with other novel proteins through various databases from building of networks of interactions. First to identify the genes which are associated with these diseases and then find the common genes are involved in all of these four diseases. For more confidence, genes contributing towards a disease were selected from various databases and then the genes which were found in most databases were selected for further process. Using these databases find several genes which are associated with the diseases and know that there are lots of genes which are involved in a single disease [206, 207].

3.3.1 Retrieval of Genes Associated with Asthma Multimorbidity, Sinusitis, Rhinosinusitis, and Gastroesophageal Reflux Disease

ENSEMBL, Polysearch and uniport were used to generate genes involved in each disease. Query with respect to each disease was given and the genes were retrieved [208].

3.3.1.1 Polysearch Approaches

A critical task in biological data mining is to extract potential associations among various kinds of biomedical objects. Poly-Search http://polysearch.ca seems to be an online system of text-mining for the identification of interactions between human pathogens, genomes, proteins, drugs, metabolites, pathogens, metabolic processes, organs, tissues, subcellular organelles, effects on health, environmental impacts, drug activity, Gene Expression words, MeSH terms, ICD-10 medical

codes, biological classification systems and chemical taxonomies. In order to improve its validity and coverage are searched by PolySearch, like uniprot, drug-Bank as well as HMDB. This database maintains a comprehensive text of biological terms and quickly retrieves related papers and database information using the latest web search technology [209]

3.3.1.2 Ensemble Approaches

Ensembl https://www.ensembl.org is a browser for the vertebrate genome that promotes the science of genetic engineering, evolution, sequence variability and transcriptional control. Currently, genes are annotated, multiple alignments are determined, predicted by regulatory activity, and data obtained from disease. The Ensemble methods involve BLAST, BioMart, BLAT and the VEP for all supported organisms. Ensemble is a forum for genome annotation and dissemination that can incorporated and summarize experimental data against reference genomes. Ensembl works for three objectives: annotation of the subphylum of the vertebrate, it allows genomic understanding, and promots research-driven study, Ensemble diversity offers compiled information from linkage results, genes association studies and genetic sources on disease-associated gene changes [210].

From ensemble get various genes of the selected genes, first to enter the diseases Asthma, Rhinosinusitis, Sinusitis and Gastroesophageal reflux disease respectively.

3.3.1.3 Uniprot Approaches

UniProt https://www.uniprot.org fundamental aim is to include a thorough coverage of a centralized protein sequence repository and an efficient approach to protein annotation, combining, evaluating, incorporating and regulating information on a wide diversity of sources [211].

It is the biggest database of protein sequences and functional annotations [212]. With the exponential increase in sequence data, it is extremely important to organize the data in an automated manner, which would provide a global genome/proteome and gene product-centric display including its sequence space that allows variations and annotations to be drilled down for each individual sequence [213].

3.3.1.4 Retrieval of Common Genes among Asthma and Comorbidities

The list was manually compared and crosschecked for the common gene identification in asthma and selected comorbidities which associated with asthma.

3.4 Identification of Pathways Shared by Asthma, Sinusitis, Rhinosi-nusitis and Gastroesophag -eal Reflux Disease

We identified a pathway that are shared between diseases between genes associated with Asthma, Sinusitis, Rhinosinusitis and Gastroesophageal Reflux disease. The pathways were retrieved and crosschecked for confirmation of genes in pathways:

1. The KEGG (Kyoto Encyclopedia of Genes and Genomes) is used to find the pathways of these diseases and find the proteins associated with disease
2. The HIPPIE (Integrating Protein Interaction Networks with Experiment) system, which includes several datasets of experimental protein-protein interaction (only HIPPIE interactions scoring > 0.63 were suggested for the HLA-A and HLA-B genes, as the authors consider them to be highly confident)
3. The Innate DB(database) server, that provides a curated collection of data based on innate immunity proteins for protein-protein interaction. In Innate DB there are 46 interaction between the genes in HLA-A gene and 35 interaction in HLA-B gene. The FIN was represented with the cytoscape.

3.4.1 KEGG Approaches

KEGG is an interactive database resource consisting of fifteen databases manually curated and four categories of a computer-generated database. PATHWAY, BRITE as well as MODULE are the databases in the device information group,

which provide the reference knowledge base for the perception of higher-level systemic cell and organism processes, including metabolism, other cellular processes, functions of organisms and diseases of humans. Another unique database is the KO database in the category of genomic details, in which knowledge of molecular functions is organized with the notion of functional orthologs [214]. In KEGG the query of HLA-A and HLA-B genes was given and associated pathways were retrieved. Pathways involving association of viral infections were discarded and only pathways related to allergy were selected. Antigen processing and presentation, phagosomes, natural killer cell regulated cytotoxicity, cell adhesion molecules and endocytosis are selected pathways.

3.4.2 HIPPIE Interactions Scoring

The HIPPIE interactions scoring ≥ 0.63 were selected for common genes playing role in asthma, sinusitis, rhinosinusitis and gastroesophageal reflux diseases by the HIPPIE table. HIPPIE incorporates multiple experimental protein-protein interaction datasets these scoring interactions extracted from the HIPPIE of HLA-A shown in Appendix and HLA-B interacting proteins in Appendix and Since the writers count them as high confidential. HIPPIE database gives interaction scoring ≥ 0.63 in both genes, after selecting the protein with assuming scoring there are a lot of genes which are taken from this database [215].

3.4.3 Proteins Interaction Data from Innate DB

Innate DB is an open database of genes, proteins, experimentally confirmed associations, and signaling pathways involved in the innate immune reaction to microbial infection in humans, mice, and bovines. Through combining established interactions and pathways from major public databases along with manually curated data into a consolidated resource, the database collects and improves the coverage of the innate immune interactome. The database could be extracted as a knowledge base or use it for system-level study of the innate immune response through certain advanced bioinformatics and visualization software [216].

Innate DB server, which provides a comprehensive collection of data about protein-protein interaction based on innate proteins of immunity. In innate DB first to find the interaction of the genes with others by entering HLA-A in INNATEDB there are 46 interactions of HLA-A genes with the other genes and after that check the interaction of HLA-B gene in the innate DB software these are 35 interaction of the HLA-B genes with the other genes. After selecting all these interactions and analyze them properly and separate the common genes in both of them and also from the other software that are used for the findings of functional interaction network [217].

3.5 Network Connectivity of Interacting Proteins with Disease-Associated Genes

Many biological procedures are focused on physical interaction between proteins. In the molecular processes regulating healthy cells, proteins function together, and studies of information on protein-protein interaction have also shown that proteins that are involved in the same disease often appear to interact among each other. The Bio-Intomics www.biointomics.com high confidence protein-protein interactions database is an excellent platform for elucidating the biological processes involved in diseases and promoting the production of drugs [218].Gene query of HLA-A and HLA-B was given default set parameter of this tool and network was retrieved.

3.6 Predicting Genes Associated With Multimorbidity

In the last step, based on established protein-disease associations, we identified the network of interacting proteins of HLA-A and HLA-B retrieved from Innate DB and HIPPIE tools was created to find their association with other proteins. Here several proteins which are predicted in the Fun-coup http://funcoup.sbc.su.se database from the selected common proteins.

3.6.1 Fun-Coup

The latest Fun-Coup release requires the entire renewal of the underlying data, such as the upgrading of multiple data sources, the inclusion of new technologies where new observational results has now become available, and the addition of Quantitative Mass Spectrometry, a new type of information data. All those proteins which were common in the interaction with HLA-A and HLA-B are further analyzed for their association with the other protein not involved or directly associated with the target diseases using the fun-coup [219].

3.7 Functional Annotation and Enrichment Analysis

Functional annotation was performed for the HLA-A and HLA-B for forming screened genes among these target diseases. [220].

3.7.1 List of Genes/Protein

Functional annotation was performed for the HLA-A and HLA-B for forming screened genes among these target diseases. First, from the pathway build before the list of genes/proteins was obtained. These genes/proteins were extracted from the Innate DB and HIPPIE Tool.

3.7.2 David And Enrich Net Tool

The David tool is the one used for the functional annotations and expression analysis. It is useful in supplying comprehensive resources to allow users to better understand biological significance in functional annotation. The list of proteins is obtained from the innateDB and HIPPIE tools into the David Tool, which provides us with clusters and P-values for functional annotation and enrichment analyses [221][226]. Same list which obtained from innateDB and HIPPIE tools was inserted into the Enrich Net tool and overlapping genes was retriverd [222].

Chapter 4

Results and Discussion

This chapter discusses in detail the results obtained from the application of the methodology as described in chapter 3. Different tools were used to address the comorbidities between the Asthma, rhinosinusitis, sinusitis and gastroesophageal reflux disease. Three allergic diseases were first selected (Asthma, rhinosinusitis and sinusitis) using literature search, in addition to these three gastroesophageal reflux diseases that is non-allergic disease was also search.

4.1 Selection of Diseases

Three allergic diseases were first selected (Asthma, rhinosinusitis and sinusitis) using literature search, in addition to these three gastroesophageal reflux diseases that is non-allergic disease was also search. In addition to these three gastroesophageal reflux diseases that is non-allergic disease was also search. While there are common mechanisms between asthma, sinusitis, rhinosinusitis and gastroesophageal reflux disease, it is still not well understood how these various mechanisms together lead to allergic and non-allergic multimorbidity. Asthma and its clinical expression may be linked to many comorbidities, but its particular effect still needs to be characterized. Comorbid conditions become more and more recognized as important determinants for asthma phenotypes and it is necessary to characterize these phenotypes.

4.2 Data Collection

A selection of genes from databases (ENSEMBL, Polysearch and Uniprot database) correlated with diseases, was built. This dataset consisted of several genes, literature was also consulted to confirm and to understand their role in diseases. It consists of all genes involved in above mentioned diseases. There are lots of genes which are involved in a single disease. We need to find the common genes related to these diseases.

4.2.1 Extraction of Genes from Polysearch

Polysearch was used to find disease-associated genes. Genes were searched by typing the name of each disease in query box. A table of searched diseases associated genes was generated. Genes related to homo sapiens were screened as a primary data. Appendix shows the result of query disease associated genes.108 genes of asthma, 3 genes of sinusitis, 17 genes of rhinosinusitis and 15 of gastroesophageal reflux disease were retrieved from polysearch.

4.2.1.1 Extraction of Genes from Ensembl

Ensembl was used to find disease-associated genes. Genes were searched by typing the name of each disease in query box. A table of searched disease associated genes was generated. Genes related to homo sapiens were screened as a primary data. Gather hundreds of genes related to asthma in human, seven genes of rhinosinusitis, almost 30 genes of sinusitis and 35 genes of gastroesophageal reflux disease were retrieved from ensemble. Appendix shows the result of query disease associated genes.

4.2.1.2 Extraction of Genes from Uniprot

Uniprot was used to find disease-associated genes. Genes were searched by typing the name of each disease in query box. A table of searched disease associated genes was generated. Genes related to homo sapiens were screened as a primary data. In the form of result extract 43 genes of asthma, 1 gene of rhinosinusitis,

32 genes of sinusitis and 15 of gastroesophageal reflux disease were retrieved from Uniprot. Appendix shows the result of query disease associated genes.

Data collected from above mentioned databases was compared and common genes in these three databases were screened for further processing.

These screened genes were common among the targeted diseases as mentioned above. These genes include HLA-A and HLA-B. After selection of these genes, associated pathways were retrieved from KEGG.

4.3 Identification of Pathways Associated with Target

The pathway is designed to recognize interactions between genes associated with Asthma, Rhinosinusitis, Sinusitis, and Gastroesophageal reflux disease. The network protein-protein interaction has been developed using the pathways of KEGG, HIPPIE and Innate DB tools. Common proteins in these repositories were used for creation of protein-protein interaction.

4.3.1 Extraction Of pathways From KEGG Related to HLA-A and HLA-B

KEGG is an interactive database resource consisting of fifteen databases manually curated and four categories of a computer-generated database. Pathways were searched by typing the name of each gene in query box. Pathways of searched diseases-associated gene was generated. Pathways related to homo sapiens were screened as a primary data. The HLA-A and HLA-B genes related 19 different pathways were retrieved, give no related to the viral genes were retrieved. Only 5 pathways which are not involved in viral or pathogens were selected. Selected pathways are:

1. Antigen processing and presentation
2. Phagosome

3. Natural killer cell mediated cytotoxicity

4. Cell adhesion molecules

5. Endocytosis

4.3.1.1 Antigen Presenting Pathway

In cell-mediated immunity, the important role played by the main histocompatibility complex (MHC) class I molecules in intracellular events such as viral infection were note, involvement of intracellular bacteria and tumor cells antigens shows in figure 4.1. They attach peptide fragments of all these proteins to the surface of the cells and present them to CD8+ T cells. This assists cytotoxic T cells to recognize cells that synthesize and eliminate abnormal or foreign substances. MHC class I consists of an invariable light chain (B2M), plus an 8-10 residual peptide ligand (B2M) and the polymer heavy chain. MHC class I is an invariable light chain (HC or alpha chain). Events in the MHC Class I biosynthesis are described here, including the generation of antigenic peptide by the mechanism of ubiquitine/26S-proteasome, the transmitting of such peptide to endoplasmatic reticulum (ER) binding to MHC Class I molecules, and showing on the cell surface of MHC Class I complexes.

4.3.1.2 Phagosome Pathway

The method by which a cell absorbs relatively large particles and is a key mechanism in restructuring, inflammation and tissue defense against pathogens is phagocytosis shows in figure 4.2. If ligands on the specific surface are recognized by the unique receptors on the phagocyte surface, a phagosome is formed. After formation, emerging phagosomes slowly evolve digestive properties. This phagosome of maturation includes controlled contact with other membrane organelles such as recycling endosomes, lysosomes and late endosomal products. The combination of phagosomes and lysosomes releases the toxic products that destroy and fragment most bacteria. Some bacteria however have strategies to resist phagocytose-related bactericidal procedures and live in hosts.

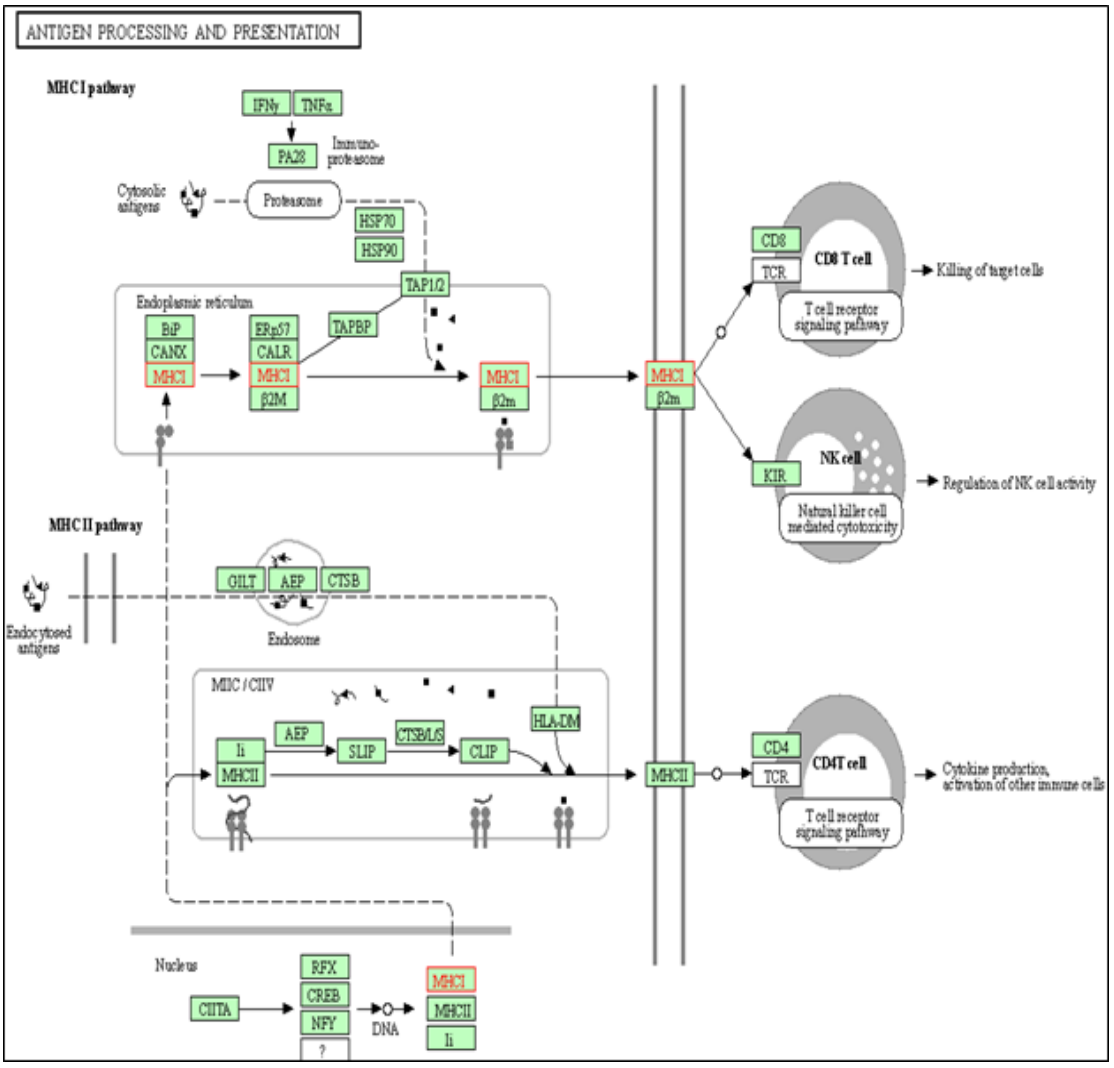


FIGURE 4.1: Antigen Presenting Presentation Pathway Extracted from KEGG

4.3.1.3 Cell Adhesion Pathway

The CAM (glycoproteins) molecules are expressed at a cellular surface and play a critical role in a broad range of biological processes including hemostasis, immune response, inflammation, embryogenesis, and neuronal tissue growth shows in figure 4.3. The integrated family, the immunoglobulin superfamily, selectins and cadherins are four principal classes. Different categories include membrane proteins that facilitate immune cell-cell interactions, including the ones involved in antigen detection, co-stimulation and cell adherence. In addition, for brain morphology and highly organized brain functions, including memory and learning, cell-cell adhesion is essential. During the early development of the nervous system, neurons extend their axons to their goals and create and preserve synapses

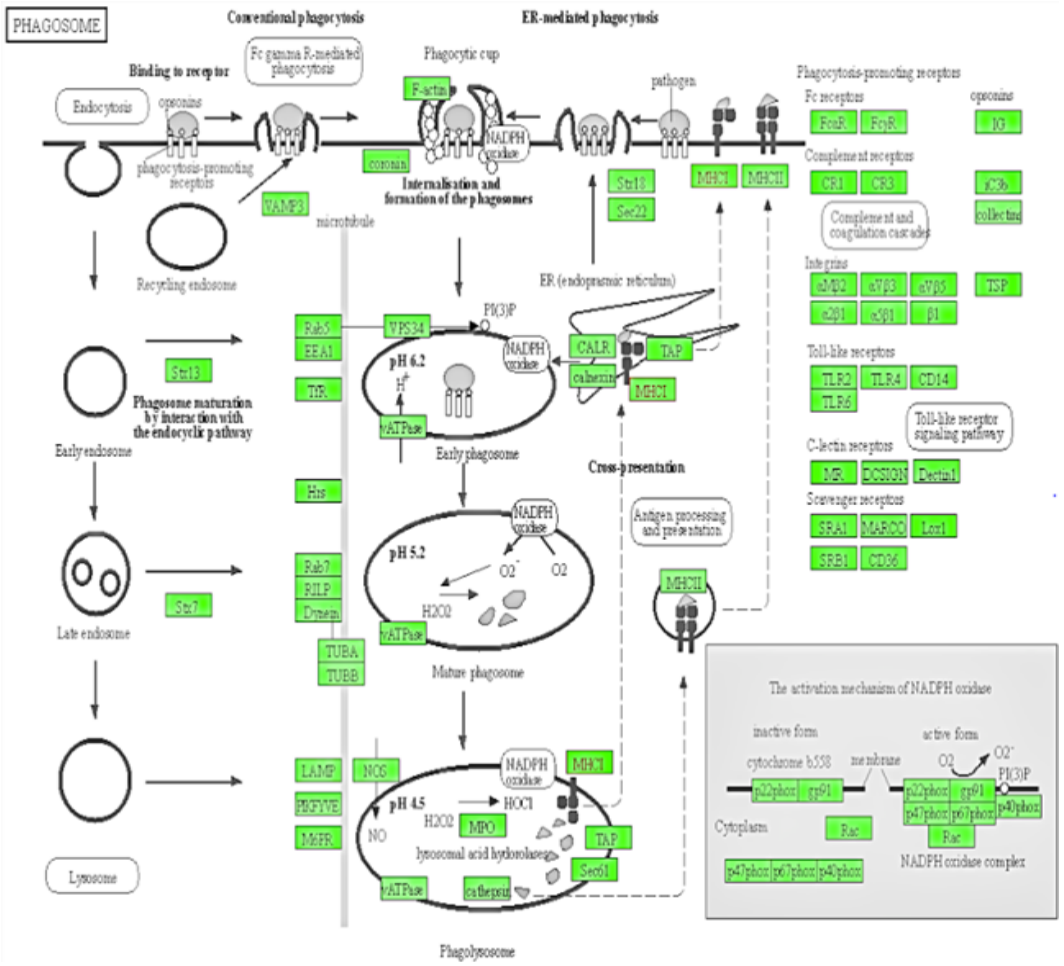


FIGURE 4.2: Phagosome Pathway Extracted from KEGG

by creating cell-cell adhesions. Cell adhesions also help axon-axon contacts and bind neurons to the swan cells assisted by oligodendrocytes.

4.3.1.4 Natural Killer Cells Pathway

NK (Natural Killer) is the innate lymphocyte of the immune system that protects both allogeneic and autologous cells under various pressures, e.g., bacterial infections, bacteria, or malignancy, from all allogeneic cells. shows in figure 4.4. While NK cells do not express classical immunoglobulin gene recipients, such as B cell antibodies or T cell receptors, they are equipped with numerous receptors whose dedication makes it possible to differentiate between cells. Cell-surface ligands are bound by active receptors, causing activation and lysis of the target cell. However, inhibitory receptors recognize the molecules of MHC class I (HLA) and

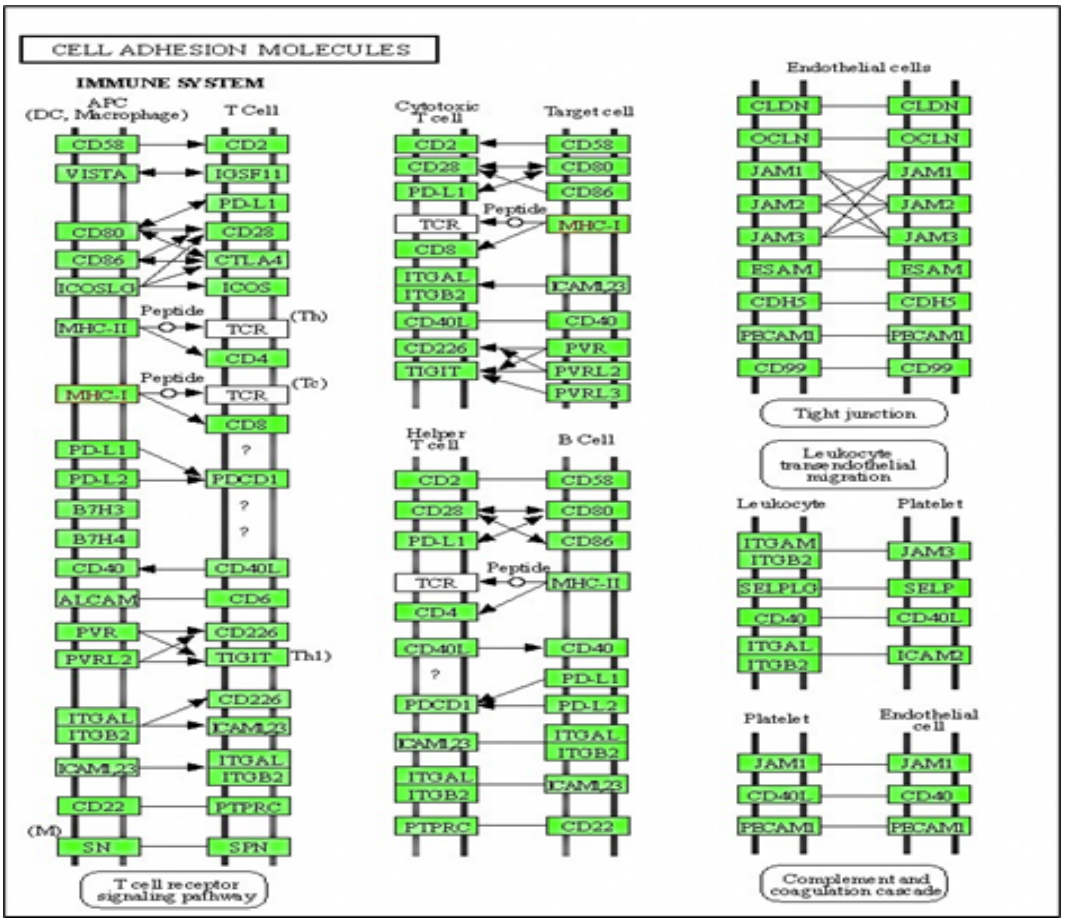


FIGURE 4.3: Cell Adhesion Molecules Pathway Extracted from KEGG

inhibit killing NK cells with the receptive activation being overruled. This inhibitory signal is lost if target cells do not express MHC class I and perhaps also in virus-infecting cells that can inhibit or modify its conformation in MHC class I. Cytotoxic granules are released to the surface and penetration into the cell membrane with effector protein and cause programmed cell deaths. The cell murder mechanism is the same as the mechanism used for cytotoxic-T-cells created by adaptive immune response.

4.3.1.5 Endocytosis Pathway

Endocytosis is a process for cells to detach from the cell surface proteins ligands, nutrients and plasma membrane (PM) that carry them into the cell interior shows in figure 4.5. Transmembrane proteins that reach the cytoplasmic domains by clathrin-dependent endocytosis (CDE) bind to the APs (adaptor-related protein

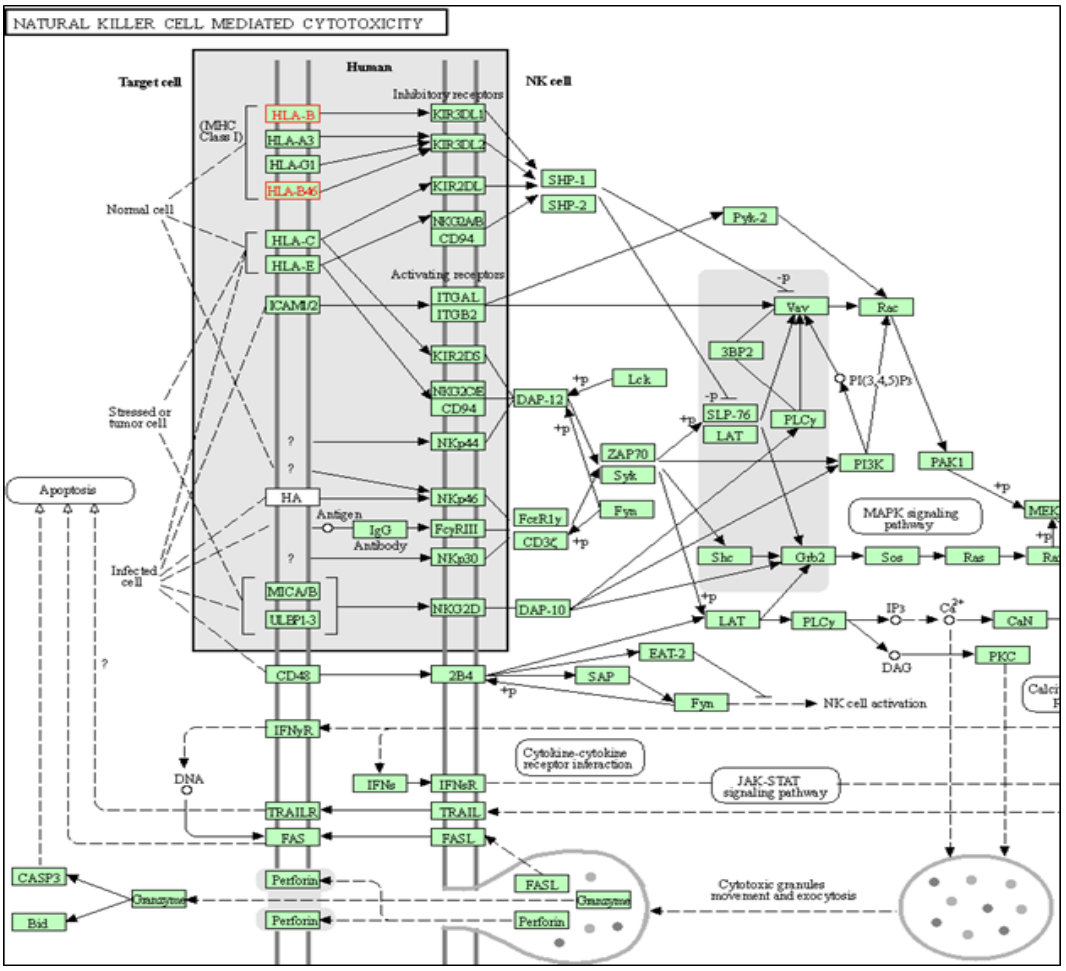


FIGURE 4.4: Natural Killer Cell Mediated Toxicity Pathway Extracted from KEGG

complexes) and enable their quick removal from PM. There are various accessory proteins including dynamine in addition to APs and clathrin. These cargoes are sorted to different destinations based on the different proteins entering the endosome membrane. Some cargoes are recycled to PM, like nutrient receptors. Ubiquities are sorted into vesicles intraluminal proteins, including activated grow-factor receptors, which eventually end up in the lysosome lumen via multivesicular endosomes (MVEs). Depending on the cargo and the cell type, there are various pathways for clathrin-dependent endocytosis (CIE).

4.3.2 Retrieval of Protein-Protein Interactions

Protein- protein interactions of selected genes (HLA-A and HLA-B) were retrieved from two databases HIPPIE and Innate DB.

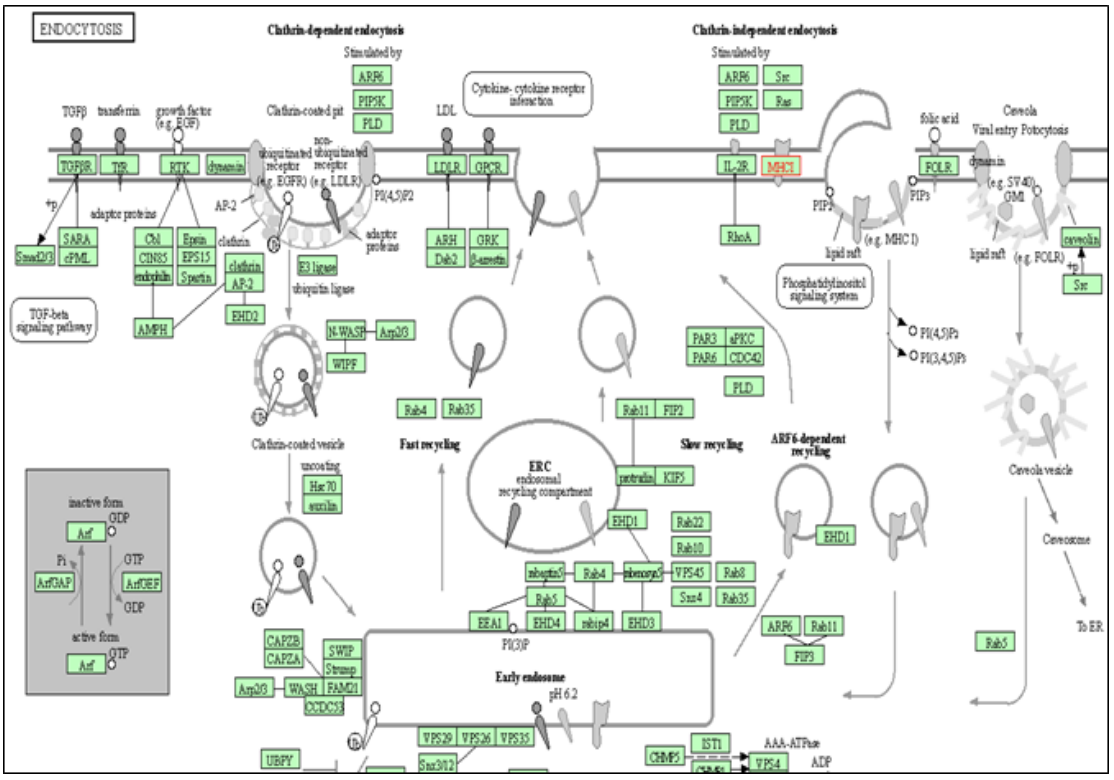


FIGURE 4.5: Natural Killer Cell Mediated Toxicity Pathway Extracted from KEGG

4.3.2.1 Retrieval of Protein from HIPPIE

The HIPPIE tool generates protein-protein interactions for the given proteins. We wanted to have all the proteins that interact with our desired proteins (HLA-A and HLA-B), as they affect system of human body. Therefore, HLA-A and HLA-B were given to HIPPIE tool which give interacting proteins for HLA-A and HLA-B. The tool gave us a large dataset for each protein therefore the threshold was set and only those interactions were selected which had high interacting score i.e., $\geq$0.63. Retrieved proteins of HLA-A and HLA-B in Appendix.

4.3.2.2 Protein-Protein Interaction Data from Innate DB

The innate DB database that keeps a compiled collection of data on protein-protein interaction. In InnateDB there were 46 interactions of HLA-A with the protein shown in Appendix and after that check the interaction of HLA-B in the innate DB software these are 35 interaction of the HLA-B with the proteins shown in Appendix.

After analyzing all these interactions, the common interactions from these two databases were used for further procedure. In the case of HLA-A 3 common proteins were selected and for HLA-B 23 common proteins were selected for construction of network.

The interaction network is designed by identifying common genes, in HLA-A gene there are KIR3DL2, KIR3DS1 and STAT3 proteins are common. In HLA-B there are B2M, CD8A, LILRB1, LILRB2, EZR, MSN, PL53, PSMD1, PAICS, RUUBLI, AHCY, VCP, TRIM28, B2M, CD8A, LILRB1, LILRB2, EZR, MSN, PL53, PSMD1, PAICS, RUUBLI, AHCY, VCP, TRIM28, ADRB2, ARHGEF4, EDEM1, HSPA5, NEDD8, PCK1, STAT1, SUM01, TRIB3 and UBD proteins are common.

4.4 Protein-Protein Network

Common interacting protein from the previous step were selected for construction of network. The networks were extract from Bio-Intomics database and found the interactions and nature of proteins with each other like protein works as a receptor, enzyme or plasma membrane bound protein.

Network shows the interaction of different proteins with the HLA-A. In figure 4.6 red symbol shows the receptor proteins. There are 9 receptor proteins which are directly interacted with HLA-A (KIR3DS1, KIR2DS4, KIR3DL2, LILRB2, LILRB1, TRBC1, TRAC, TRAV21 and TRBV6-5) and their number of interacting proteins in table 4.1. Sky blue sign shows the enzyme proteins in which HLA-A also directly interact with 5 enzymes called SYVN1, TMEM129, UBE2J1, UBE2J2 and PDIA3 and their interacting proteins number shown in table 4.6. Orange sign shows the plasma membrane bound proteins in which 11 plasma membrane bound protein is directly involved with HLA-A these SELENOS, PMEL, MME, BCAP31, HLA-DRB1, HLA-F, TRB, TRA, CD8A, HLA-DRA and TAPBPL, number of interacting proteins shown in table 4.3. Circle sign shows the 24 interacting predicted proteins which directly interact with the HLA-A but function is unknown as mention in Bio- Intomics. HLA-A itself a plasma membrane bound protein.

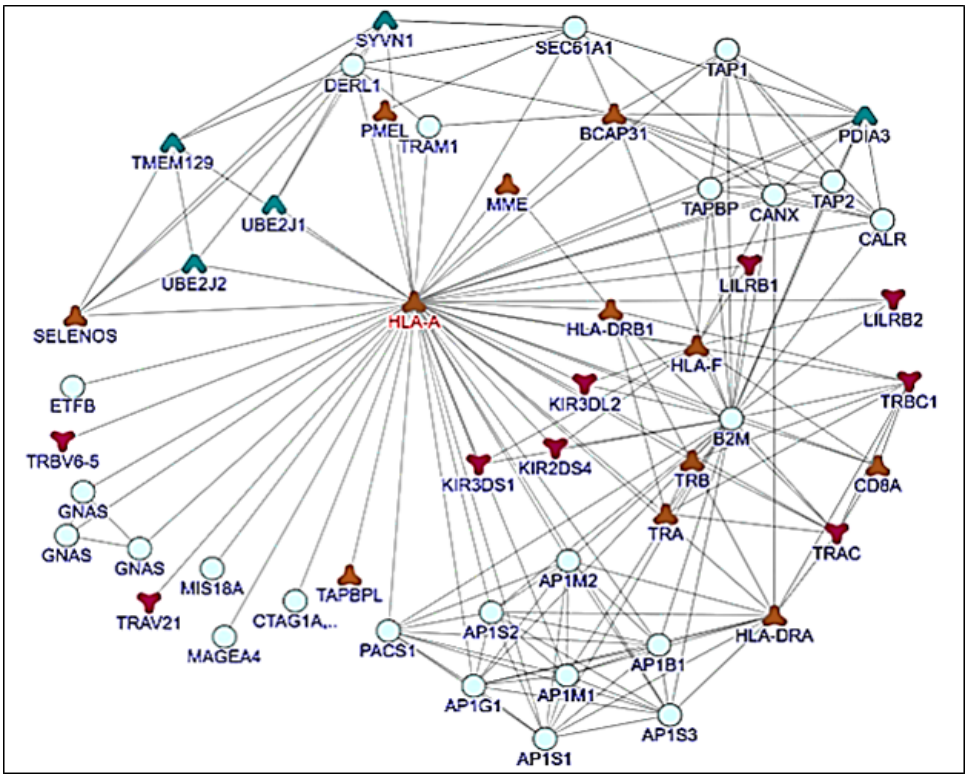


FIGURE 4.6: HLA-A Protein Interaction Network Extracted from The Bio-Intomics

TABLE 4.1: HLA-A gene interactions with Receptors and their functions.

Sr	Name of genes/proteins	No. of interacting proteins	Name of interacting proteins
1.	KIR3DS1	3	HLA-F, HLA-A, B2M
2.	KIR2DS4	3	HLA-F, HLA-A, B2M
3.	KIR3DL2	3	HLA-F, HLA-A, B2M
4.	LILRB2	4	INS, HLA-A, HLA-F,B2M
5.	LILRBI	4	INS, HLA-A, HLA-F, B2M
6.	TRBCI	8	HLA-DRB1, HLA-A, TRB, M2M, TRA, TRAC, HLA-DRA, CD84
7.	TRAC	8	HLA-DRB1, HLA-A, TRB, B2M, CD84, TRA, TRACI, HLA-DRA
8.	TRAV2I	1	HLA-A
9.	TRBV6-5	1	HLA-A

TABLE 4.2: HLA-A gene interactions with Enzymes and their functions

Sr	Name of genes/proteins	No. of interacting proteins	Name of interacting proteins
1.	SYVNI	8	SELENOS, HLAA, SEC6IAI, BAG6, VCP, DERL1, UBE2J1, TMEMI29
2.	VCP	11	SEC6IAI, BAC6, HLA-DRBI, HLAA, DERLI, UBE2J2, SYVNI, SELENOS, TEMEMI29, HSP9CAAI, UBE2JI
3.	TMEMI29	6	VCP, HLA-A, SYVNI, DER2, UBE2J2, SELENOS
4.	UBE2J1	4	VCP, HLA-A, SYVNI, DERLI
5.	UBE2J2	5	TMEMI29, SELENOS, VCP, HLA-A, DERLI
6.	PDIA3	9	SEC6IAI, HLAA, BCAP31, TAPI, TAPBP, CANX, TAPL, CALR, B2M

TABLE 4.3: HLA-A gene interactions with Plasma membrane bound and their functions

Sr	Name of genes/proteins	No. of interacting proteins	Name of interacting proteins
1.	SELENOS	6	HLAA, DERL1, VCP, UBE2J2, TMEMI29, SYVN1
2.	PMEL	2	HLA-A, SEC61A1
3.	MME	3	HLA-A, CD9, HLA-DRB1

4.	CD9	3	MME, HLA-A, HLA-DRBI
5.	BCAP31	11	SEC61A1, TRAM1, DERL1, HLAA, TAP1, TAPBP, CALR, PDIA3, CANX, B2M, TAP2
6.	HLA-DRBI	8	TRBC1, TRAC, HLA-DRA, TRA, HLA-A, MME, CD9, VCP
7.	HLA-F	10	CD8A, KIR3DSI, B2M, KIR2DS4, KIR3DL2, HLA-A, TAPBP, LILRB2, LILRBI, CANX
8.	TRB	5	TRA, TRAC, TRACI, HLA-A, B2M
9.	TRA	6	HLA-A, HLA-DRB1, B2M, TRBCI, TRAC, TRB
10.	CD8A	5	HLA-A, HLA-F, B2M, TRBCI, TRAC
11.	HLA-DRA	12	APIB1, AP1SB, APISI, APISI, AP1MI, APIS2, APIMI, HLA-A, TRAC, TRACI, CANX, HLA-DRBI
12.	TAPBPL	1	HLA-A
13.	MEPIB	1	HLA-A

Network shows the interaction of different proteins with the HLA-B. In figure 4.7 red symbol shows the receptor proteins. There are 12 receptor proteins which are directly interacted with HLA-B (KIR3DS1, KIR2DS4, KIR3DL2, KIR2DL3, KIR2DL1, KIR3DL1, LILRB2, LILRB1, LILRA1, TRBC1, TRAC, and CXCR4) and their interacting proteins shown in Table 4.4. Sky blue sign shows the enzyme

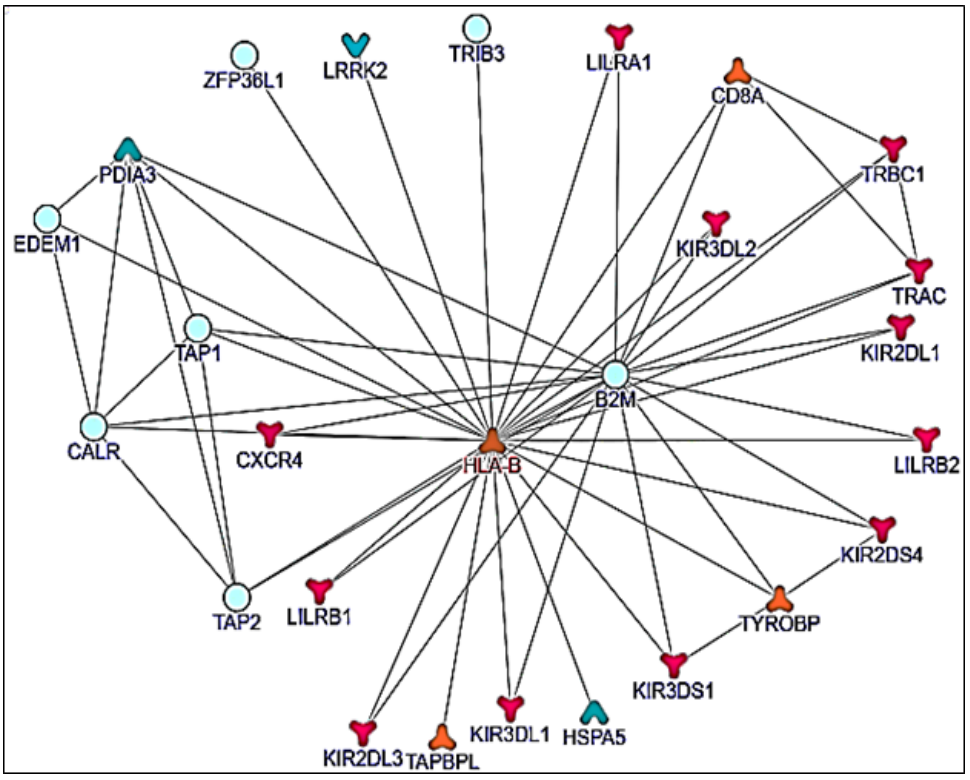


FIGURE 4.7: HLA-B Protein Interaction Network Extracted from The Bio-Intomics

proteins in which HLA-B also directly interact with 3 enzymes called LRRK2, HSPA5and PDIA3, interacting proteins are shown in Table 4.5. Orange sign shows the plasma membrane bound proteins in which 3 plasma membrane bound protein is directly involved with HLA-B these TYROBP, CD8A and TAPBPL. Circle sign shows the 7 interacting proteins with directly interact with the HLA-B but function is unknown as mention in Bio-Intomics. HLA-B itself a plasma membrane bound protein.

TABLE 4.4: HLA-B gene interactions with Receptor and their functions.

Sr	**Name of genes/proteins**	**No. of interacting proteins**	**Name of interacting proteins**
1.	LILRA1	2	HLA-B, B2M
2.	KIR3DL2	2	HLA-B, B2M
3.	TRBCI	4	HLA-B, B2M, CD8A, TRAC
4.	TRAC	4	HLA-B, B2M, CD8A, TRBC1

5.	KIR2DLI	2	HLA-B, B2M
6.	LILRB2	2	HLA-B, B2M
7.	KIR2DS4	3	HLA-B, B2M, TYROBP
8.	KIR3DSI	3	HLA-B, B2M, TYROBP
9.	KIR3DLI	2	HLA-B, B2M
10.	KIR2DL3	2	HLA-B, B2M
11.	LILRBI	2	HLA-B, B2M
12.	CXCR4	2	HLA-B, B2M

Table 4.5: HLA-B gene interactions with Enzymes and their functions.

Sr	**Name of genes/proteins**	**No. of interacting proteins**	**Name of interacting proteins**
1.	CD8A	4	HLAB, B2M, TRAC, TRBC1
2.	TAPBPL	1	HLA-A
3.	TYROBP	4	HLA-B, B2M, KIR3DSI, KIR2DS4

Table 4.6: HLA-B gene interactions with Enzymes and their functions

Sr	**Name of genes/proteins**	**No. of interacting proteins**	**Name of interacting proteins**
1.	PDIA3	6	HLA-B-B2M, EDEM1, CALR, TAP11, TAP2
2.	HSPA5	1	HLA-B

4.5 Protein-Protein Interaction of Common Proteins Screened From KEGG, HIPPIE and InnateDB

All those proteins which were common in the interaction with HLA-A and HLA-B are further analyzed for their association with the other protein not involved or

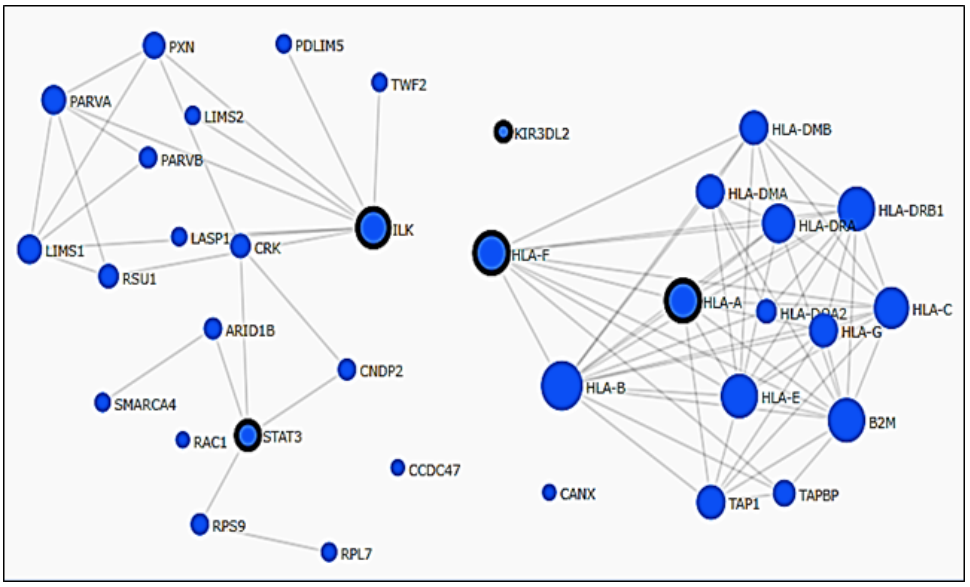


FIGURE 4.8: HLA-A Interacting protein interaction taking from Funcoup

directly associated with the target diseases using the fun-coup as mentioned in figure 4.8 and 4.9.

Network of all those common proteins which were interacting with the HLA-A is shown in the figure 4.8. In this diagram there are 2 type of circle visualization one is simple blue and the second one is blue with thick black boundary. The circle which are highlighted with thick black colour are proteins of interest and blue color circles represent those proteins which are interacting with our proteins of interest. KIR3DL2 target protein did not show any interaction but it shows the interaction network generated for the interaction of HLA-A with the proteins screened from the KEGG pathway, innate DB and HIPPIE (figure 4.4). ILK and STAT3 do not show the interaction with HLA-A and similar results were also generated when HLA-A network was generated. Although these proteins were common in selected diseases.

Network of all those common proteins which were interacting with HLA-B is shown in the figure 4.9. All the genes are interacting with each other. No gene were single and self-interacted. similar results were also generated when HLA-B network was generated. Although these proteins were common in selected diseases. This data can be translated and combined into protein association networks where proteins represent nodes and connections represent associations.

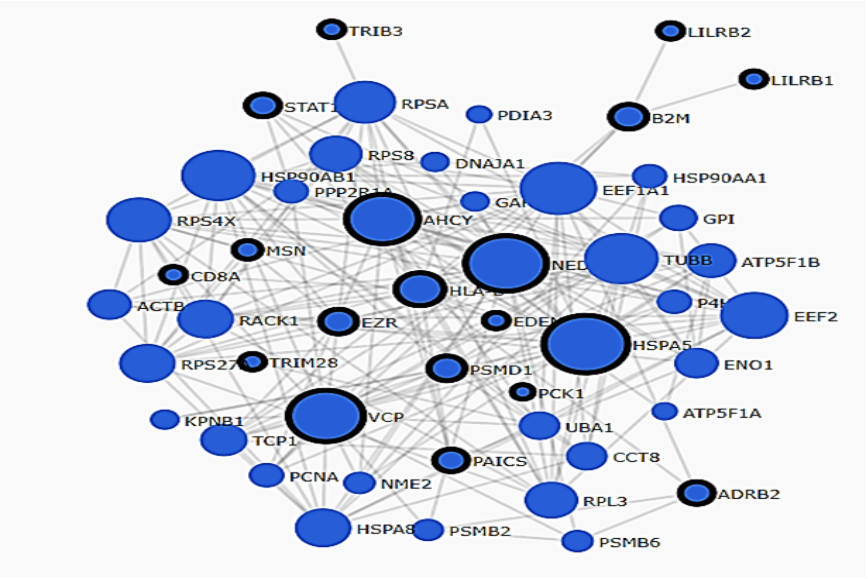


FIGURE 4.9: HLA-B Interacting genes interaction taking from Funcoup.

4.6 Prediction of Proteins from the Multimorbidity Diseases-Associated Genes

4.6.1 HLA-A Predicted Proteins

PARVA, PARVB, PXN, LIMS2, LIMS1, RSU1, LASP1, CRK, PDLIM5, TWF2, RAC1, RPS9, RPL7 and CNDP2 are the proteins which are closely interacted to the multimorbidity common disease-associated genes with HLA-A gene.

4.6.2 HLA-B Predicted Proteins

PSMB6, PSMB2, RPL3, HSPA8, NME2, PCNA, TCP1, KPNB1, UBA1, ATP5 F1A, ENO1, EEF2, ATP5 F1B, TUBB, NED, RACK1, ACTB, RPS27, RPS4X, RPSA, EEF1A1, GAT1, PPP2 B1A, RPSA, DNAJA1, GP1 and TUBB PSMB6, PSMB2, RPL3, HSPA8, NME2, PCNA, TCP1, KPNB1, UBA1, ATP5F1A, ENO1, EEF2, ATP5 F1B, TUBB, NED, RACK1, ACTB, RPS27, RPS4X, RPSA, EEF1 A1, GAT1, PPP2 B1A, RPSA, DNA JA1, GP1 and TUBB are the proteins which are closely interacted to the multimorbidity common disease associated genes HLA-B genes.

4.7 Functional Annotation and Enrichment Analysis

The genes/proteins work in a particular pathway to perform their function and to produce a particular response. For the communication to build cells pathways genes/proteins are often overlapping in the other pathway that is any change on one pathway tends to produce change effect in the other which is called crosstalk [223].

All common four proteins for HLA-A and 21 proteins for HLA-B that were retrieved from Innate DB, HIPPIE and KEGG were used to find the crosstalk.

TABLE 4.7: List of proteins interacting with HLA-A and HLA-B with their IDs

Diseases Name	Common Genes	Interacting Proteins	Gene ID
	HLA-A	HLA-A	3105
		KIR3DL2	3812
		KIR3DS1	3813
		STAT3	6774
	HLA-B	HLA-B	3106
		B2M	567
		CD8A	925
		LILRB1	10859
		LILRB2	10288
		EZR	7430
Asthma		MSN	4478
Rhinosinusitis		PSMD1	5707
Sinusitis		PAICS	10606
Gastroesophageal		AHCY	191
reflux disease		VCP	7415
		TRIM28	10155
		ADRB2	154
		ARHGEF4	50649

EDEM1	9695
HSPA5	3309
NEDD8	4738
PCK1	5105
STAT1	6772
TRIB3	57761
UBD	10537

4.7.1 Functional Annotation by David Tool

David database was used to perform functional annotation. The results obtained after functional annotation were in the form of clusters. Functional categories based on a co-existence with a group of genes will easily help to unravel new bio-pathway processes. If genes share similar set of those terms, they are most likely involved in similar biological mechanisms [220].

The result of David tool gave us different clusters, we selected 1 cluster for HLA-A and 7 clusters of HLA-B by the enrichment score is $\geq$1 and p value as $\leq$0.1 as threshold.

4.7.1.1 HLA-A Functional Annotation Clustering

After functional annotation, 1 cluster of interacting HLA-A proteins was generated. The enrichment score was 1.17. The functions of these genes are involved in immunoglobin domain, plasma membrane, integral component of plasma membrane, disulphide bond signal peptide, topological domain(extracellular), glycosylation site, glycoprotein, transmembrane region, polymorphism, sequence variant and alternative splicing.

The results in figure 4.10 indicates HLA-A, KIR3DL2, KIR3DS1 and STAT3 forms one cluster and plays role in plasma membrane, the enrichment score (1.17) and p-value (0.01) indicate that these proteins are not clustered accidentally rather they are significantly more enriched.

Annotation Cluster 1	Enrichment Score: 1.17			Count	P_Value	Benjamini
UP_KEYWORDS	Immunoglobulin domain	RT		3	2.1E-3	6.0E-2
INTERPRO	Immunoglobulin-like domain	RT		3	5.0E-3	8.0E-2
INTERPRO	Immunoglobulin-like fold	RT		3	7.6E-3	8.0E-2
GOTERM_CC_DIRECT	plasma membrane	RT		4	1.2E-2	2.1E-1
GOTERM_CC_DIRECT	integral component of plasma membrane	RT		3	1.7E-2	2.1E-1
UP_SEQ_FEATURE	topological domain:Extracellular	RT		3	5.3E-2	4.6E-1
UP_SEQ_FEATURE	disulfide bond	RT		3	5.7E-2	4.6E-1
UP_SEQ_FEATURE	signal peptide	RT		3	7.4E-2	4.6E-1
UP_KEYWORDS	Disulfide bond	RT		3	7.4E-2	7.1E-1
UP_SEQ_FEATURE	topological domain:Cytoplasmic	RT		3	7.9E-2	4.6E-1
UP_KEYWORDS	Signal	RT		3	1.1E-1	7.1E-1
UP_SEQ_FEATURE	glycosylation site:N-linked (GlcNAc...)	RT		3	1.1E-1	5.9E-1
UP_KEYWORDS	Glycoprotein	RT		3	1.3E-1	7.1E-1
UP_SEQ_FEATURE	transmembrane region	RT		3	1.6E-1	7.2E-1
UP_KEYWORDS	Transmembrane helix	RT		3	1.8E-1	7.1E-1
UP_KEYWORDS	Transmembrane	RT		3	1.8E-1	7.1E-1
GOTERM_CC_DIRECT	integral component of membrane	RT		3	2.0E-1	1.0E0
UP_KEYWORDS	Polymorphism	RT		4	2.0E-1	7.1E-1
UP_SEQ_FEATURE	sequence variant	RT		4	2.4E-1	9.8E-1
UP_KEYWORDS	Membrane	RT		3	3.0E-1	8.7E-1
UP_KEYWORDS	Alternative splicing	RT		3	5.2E-1	1.0E0

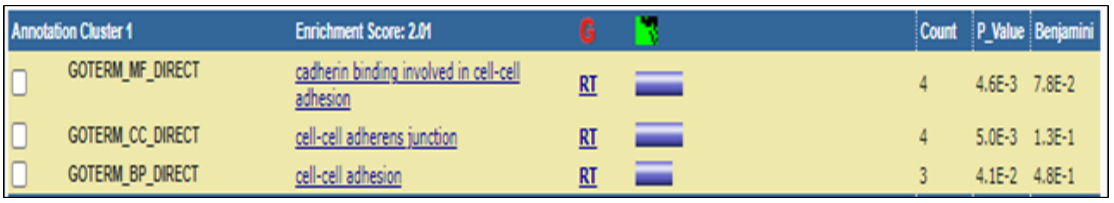
FIGURE 4.10: Functional annotation of HLA-A associated genes

4.7.1.2 HLA-B Functional Annotation Clustering

7 clusters of HLA-B interacting genes were found out after functional annotation. The highest enrichment score was 2.01 and the lowest being 0.45.

Annotation Cluster 1

Annotation Cluster 1 of HLA-B interacting genes were found out after functional annotation. The functions of these genes are involved in cadherin binding involved in cell-cell adhesion, cell-cell adherens junction and cell-cell adhesion. The results in figure 4.11 indicates EZR, HSPA5, PAICS and STAT1 forms cluster and plays role in cell-cell adherens junction, the enrichment score (2.01) and p-value (0.004) show that these proteins are not mistakenly clustered but are considerably enriched.

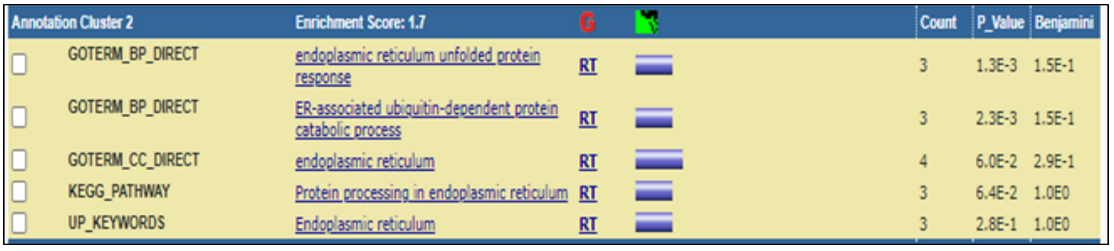


Annotation Cluster 1	Enrichment Score: 2.01			Count	P_Value	Benjamini
GOTERM_MF_DIRECT	cadherin binding involved in cell-cell adhesion	RT		4	4.6E-3	7.8E-2
GOTERM_CC_DIRECT	cell-cell adherens junction	RT		4	5.0E-3	1.3E-1
GOTERM_BP_DIRECT	cell-cell adhesion	RT		3	4.1E-2	4.8E-1

FIGURE 4.11: Functional annotation of HLA-B associated genes

Annotation Cluster 2

The HLA-B interacting genes annotation cluster 2 were identified after functionally annotated genes. The enrichment score were 1.7. The functions of these genes are involved in endoplasmic reticulum unfolded protein response, ER -associated ubiquitin-dependent protein catabolic process, endoplasmic reticulum and protein processing in endoplasmic reticulum. The results in figure 4.12 indicates EDEM1, HSPA5, HLA-B and VCP forms cluster and plays role in endoplasmic reticulum, p-value (0.06) show that these proteins are not mistakenly clustered and are considerably significant.



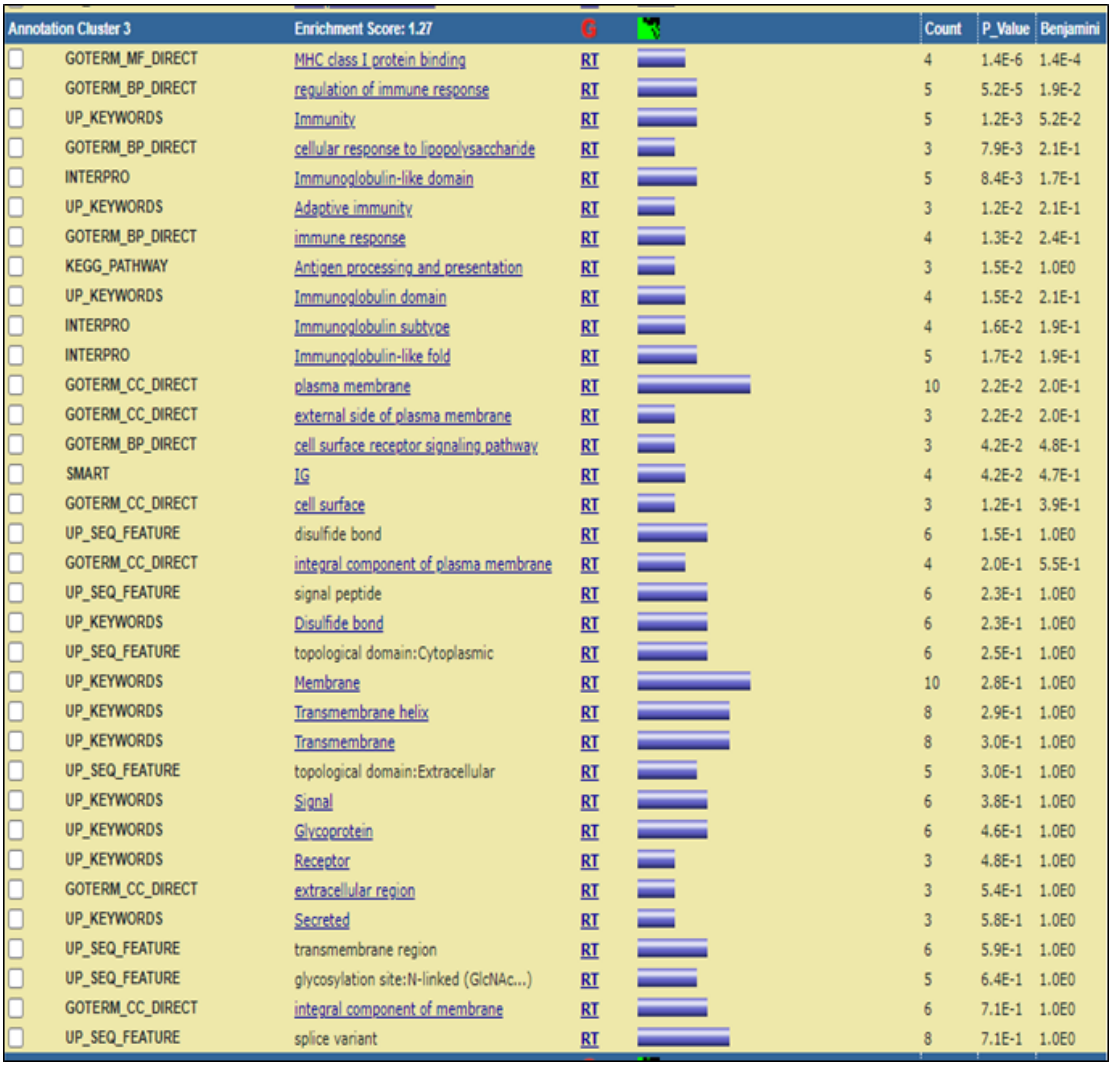
Annotation Cluster 2	Enrichment Score: 1.7			Count	P_Value	Benjamini
GOTERM_BP_DIRECT	endoplasmic reticulum unfolded protein response	RT		3	1.3E-3	1.5E-1
GOTERM_BP_DIRECT	ER-associated ubiquitin-dependent protein catabolic process	RT		3	2.3E-3	1.5E-1
GOTERM_CC_DIRECT	endoplasmic reticulum	RT		4	6.0E-2	2.9E-1
KEGG_PATHWAY	Protein processing in endoplasmic reticulum	RT		3	6.4E-2	1.0E0
UP_KEYWORDS	Endoplasmic reticulum	RT		3	2.8E-1	1.0E0

FIGURE 4.12: Functional annotation of HLA-B associated genes

Annotation Cluster 3

Annotation cluster 3 of HLA-B interacting genes were found out after functional annotation. The enrichment score were 1.27. The functions of these genes are involved in MHC class 1 protein binding, regulation of immune response, immunity, cellular response to lipopolysaccharide, Immunoglobulin-like domain, adaptive immunity, immune response, antigen processing and presentation, plasma membrane, external side of plasma membrane, cell surface receptor signaling pathway, IG, cell surface, disulfide bond, integral component of plasma membrane, signal peptide, topological domain, transmembrane , glycoprotein, receptor extracellular regions,

secreted, glycosylation site and splice variants. The results in figure 4.13 indicates CD8A, ADRB2, B2M, EZR, HSPA5, LILRB1, LILRB2, HLA-B, MSN and TRIB3 forms cluster and plays role in plasma membrane, p-value (0.02) Show that these proteins are not clustered mistakenly, but enriched significantly.



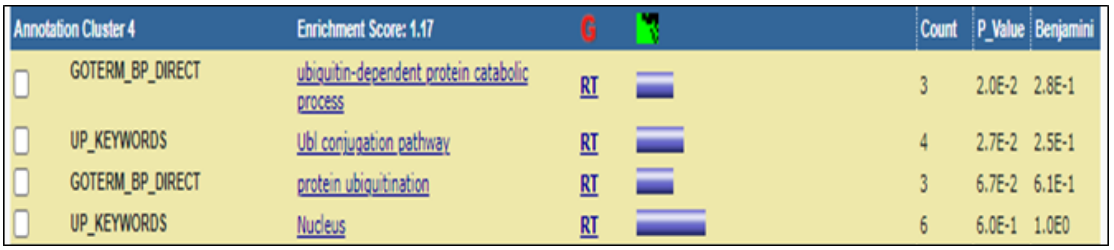
Annotation Cluster 3	Enrichment Score: 1.27	G		Count	P_Value	Benjamini
GOTERM_MF_DIRECT	MHC class I protein binding	RT		4	1.4E-6	1.4E-4
GOTERM_BP_DIRECT	regulation of immune response	RT		5	5.2E-5	1.9E-2
UP_KEYWORDS	Immunity	RT		5	1.2E-3	5.2E-2
GOTERM_BP_DIRECT	cellular response to lipopolysaccharide	RT		3	7.9E-3	2.1E-1
INTERPRO	Immunoglobulin-like domain	RT		5	8.4E-3	1.7E-1
UP_KEYWORDS	Adaptive immunity	RT		3	1.2E-2	2.1E-1
GOTERM_BP_DIRECT	immune response	RT		4	1.3E-2	2.4E-1
KEGG_PATHWAY	Antigen processing and presentation	RT		3	1.5E-2	1.0E0
UP_KEYWORDS	Immunoglobulin domain	RT		4	1.5E-2	2.1E-1
INTERPRO	Immunoglobulin subtype	RT		4	1.6E-2	1.9E-1
INTERPRO	Immunoglobulin-like fold	RT		5	1.7E-2	1.9E-1
GOTERM_CC_DIRECT	plasma membrane	RT		10	2.2E-2	2.0E-1
GOTERM_CC_DIRECT	external side of plasma membrane	RT		3	2.2E-2	2.0E-1
GOTERM_BP_DIRECT	cell surface receptor signaling pathway	RT		3	4.2E-2	4.8E-1
SMART	IG	RT		4	4.2E-2	4.7E-1
GOTERM_CC_DIRECT	cell surface	RT		3	1.2E-1	3.9E-1
UP_SEQ_FEATURE	disulfide bond	RT		6	1.5E-1	1.0E0
GOTERM_CC_DIRECT	integral component of plasma membrane	RT		4	2.0E-1	5.5E-1
UP_SEQ_FEATURE	signal peptide	RT		6	2.3E-1	1.0E0
UP_KEYWORDS	Disulfide bond	RT		6	2.3E-1	1.0E0
UP_SEQ_FEATURE	topological domain:Cytoplasmic	RT		6	2.5E-1	1.0E0
UP_KEYWORDS	Membrane	RT		10	2.8E-1	1.0E0
UP_KEYWORDS	Transmembrane helix	RT		8	2.9E-1	1.0E0
UP_KEYWORDS	Transmembrane	RT		8	3.0E-1	1.0E0
UP_SEQ_FEATURE	topological domain:Extracellular	RT		5	3.0E-1	1.0E0
UP_KEYWORDS	Signal	RT		6	3.8E-1	1.0E0
UP_KEYWORDS	Glycoprotein	RT		6	4.6E-1	1.0E0
UP_KEYWORDS	Receptor	RT		3	4.8E-1	1.0E0
GOTERM_CC_DIRECT	extracellular region	RT		3	5.4E-1	1.0E0
UP_KEYWORDS	Secreted	RT		3	5.8E-1	1.0E0
UP_SEQ_FEATURE	transmembrane region	RT		6	5.9E-1	1.0E0
UP_SEQ_FEATURE	glycosylation site:N-linked (GlcNAc...)	RT		5	6.4E-1	1.0E0
GOTERM_CC_DIRECT	integral component of membrane	RT		6	7.1E-1	1.0E0
UP_SEQ_FEATURE	splice variant	RT		8	7.1E-1	1.0E0

FIGURE 4.13: Functional annotation of HLA-B associated genes

Annotation Cluster 4

Annotation cluster 4 of HLA-B interacting genes were found out after functional annotation. The enrichment score was 1.17. The functions of these genes are involved in ubiquitin-dependent protein catabolic process, ubl conjugation pathway, protein ubiquitination and nucleus. The results in figure 4.14 indicates NEDD8, STAT1, TRIB3, TRIM28, UBD and VCP forms cluster and plays role in nucleus,

p-value (0.2) Demonstrate that these proteins are not accidently clustered and greatly enriched.

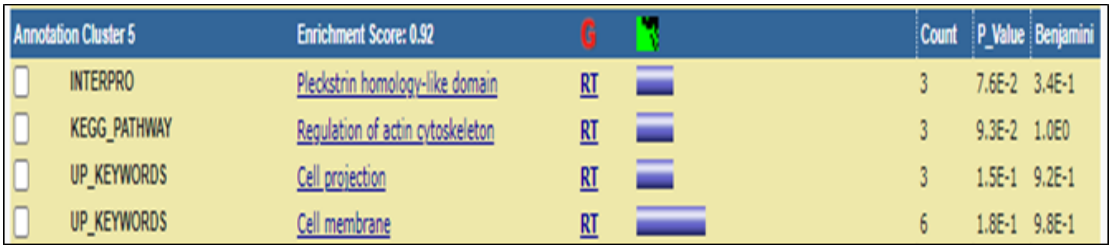


Annotation Cluster 4	Enrichment Score: 1.17			Count	P_Value	Benjamini
GOTERM_BP_DIRECT	ubiquitin-dependent protein catabolic process	RT		3	2.0E-2	2.8E-1
UP_KEYWORDS	Ubl conjugation pathway	RT		4	2.7E-2	2.5E-1
GOTERM_BP_DIRECT	protein ubiquitination	RT		3	6.7E-2	6.1E-1
UP_KEYWORDS	Nucleus	RT		6	6.0E-1	1.0E0

FIGURE 4.14: Functional annotation of HLA-B associated genes

Annotation Cluster 5

Annotation cluster 5 of HLA-B interacting genes were found out after functional annotation. The enrichment score was 0.92(non significant). The functions of these genes are involved in pleckstrin homology-like domain, regulation of actin cytoskeleton, cell projection and cell membrane. The results in figure 4.15 indicates CD8A, ARHGEF4, ADRB2, EZR, LILRB1, CD8A, ARHGEF4, ADRB2, EZR, LILRB1 and MSN forms cluster and plays role in cell membrane, p-value (0.1) indicate that these proteins are significantly clustered.

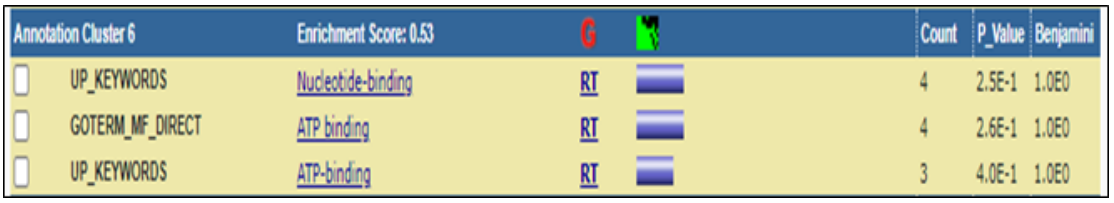


Annotation Cluster 5	Enrichment Score: 0.92			Count	P_Value	Benjamini
INTERPRO	Pleckstrin homology-like domain	RT		3	7.6E-2	3.4E-1
KEGG_PATHWAY	Regulation of actin cytoskeleton	RT		3	9.3E-2	1.0E0
UP_KEYWORDS	Cell projection	RT		3	1.5E-1	9.2E-1
UP_KEYWORDS	Cell membrane	RT		6	1.8E-1	9.8E-1

FIGURE 4.15: Functional annotation of HLA-B associated genes

Annotation Cluster 6

Annotation cluster 6 of HLA-B interacting genes were found out after functional annotation. The enrichment score was 0.53 (non-significant). The enrichment score was 0.53 (non-significant). The functions of these genes are involved in nucleotide-binding and ATP-binding. The results in figure 4.16 indicates HSPA5, PCK1, PAICS and VCP forms cluster and plays role in nucleotide binding, p-value (0.2) Show that these proteins are clustered mistakenly, non-significant.

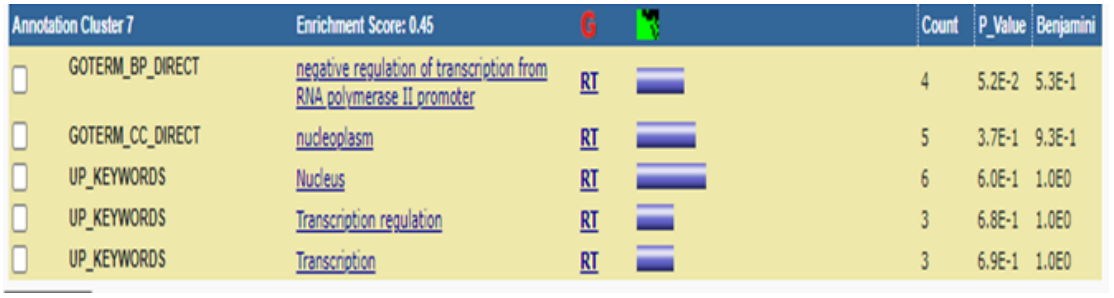


Annotation Cluster 6	Enrichment Score: 0.53			Count	P_Value	Benjamini
UP_KEYWORDS	Nucleotide-binding	RT		4	2.5E-1	1.0E0
GOTERM_MF_DIRECT	ATP binding	RT		4	2.6E-1	1.0E0
UP_KEYWORDS	ATP-binding	RT		3	4.0E-1	1.0E0

FIGURE 4.16: Functional annotation of HLA-B associated genes

Annotation Cluster 7

Annotation cluster 7 of HLA-B interacting genes were found out after functional annotation. The enrichment score was 0.45(non-significant). The functions of these genes are involved in negative regulation of transcription from RNA polymerase 2 promotor, nucleoplasm, nucleus, transcription regulation and transcription. The results in figure 4.17 indicates NEDD8, STAT1, TRIB3, TRIM28, UBD and VCP forms cluster and plays role in nucleus, p-value (0.05) Present that certain proteins are not mistakenly clustered and have significant importance.



Annotation Cluster 7	Enrichment Score: 0.45			Count	P_Value	Benjamini
GOTERM_BP_DIRECT	negative regulation of transcription from RNA polymerase II promoter	RT		4	5.2E-2	5.3E-1
GOTERM_CC_DIRECT	nucleoplasm	RT		5	3.7E-1	9.3E-1
UP_KEYWORDS	Nucleus	RT		6	6.0E-1	1.0E0
UP_KEYWORDS	Transcription regulation	RT		3	6.8E-1	1.0E0
UP_KEYWORDS	Transcription	RT		3	6.9E-1	1.0E0

FIGURE 4.17: Functional annotation of HLA-B associated genes

4.7.1.3 Enrichment Analysis

TABLE 4.8: functional annotation were done which shows no of different overlapping genes

Pathway/Process	**Gene set size**	**Pathway size**	**Overlap Genes**
hsa04612: Antigen processing and presentation	24	66	HSPA5 KIR3DS1 KIR3DL2 B2M CD8A

hsa05332: Graft-versus-host disease	24	40	KIR3DS1 KIR3DL2
hsa04964: Proximal tubule bicarbonate reclamation	24	21	PCK1
hsa03060: Protein export	24	23	HSPA5
hsa00450: Selenoamino acid metabolism	24	25	AHCY
hsa00020: Citrate cycle (TCA cycle)	24	30	PCK1
hsa05340: Primary immunodeficiency	24	33	CD8A
hsa04920: Adipocytokine signaling pathway	24	67	PCK1 STAT3
hsa00270: Cysteine and methionine metabolism	24	34	AHCY
hsa05212: Pancreatic cancer	24	70	STAT1 STAT3
hsa05020: Prion diseases	24	35	HSPA5
hsa00620: Pyruvate metabolism	24	40	PCK1
hsa03050: Proteasome	24	43	PSMD1
hsa05130: Pathogenic Escherichia coli infection	24	52	EZR

hsa04141: Protein processing in endoplasmic reticulum	24	157	HSPA5 VCP EDEM1
hsa05221: Acute myeloid leukemia	24	54	STAT3
hsa04670: Leukocyte transendothelial migration	24	113	MSN EZR
hsa00010: Glycolysis / Gluconeogenesis	24	62	PCK1
hsa04650: Natural killer cell mediated cytotoxicity	24	130	KIR3DS1 KIR3DL2
hsa03320: PPAR signaling pathway	24	69	PCK1
hsa04810: Regulation of actin cytoskeleton	24	208	MSN EZR ARHGEF4
hsa04971: Gastric acid secretion	24	71	EZR
hsa05140: Leishmaniasis	24	72	STAT1
hsa04630: Jak-STAT signaling pathway	24	154	STAT1 STAT3
hsa04970: Salivary secretion	24	83	ADRB2
hsa04640: Hematopoietic cell lineage	24	87	CD8A
hsa04062: Chemokine signaling pathway	24	186	STAT1 STAT3

hsa04620: Toll-like receptor signaling pathway	24	101	STAT1
hsa04660: T cell receptor signaling pathway	24	108	CD8A
hsa04514: Cell adhesion molecules (CAMs)	24	131	CD8A
hsa04910: Insulin signaling pathway	24	134	PCK1
hsa00230: Purine metabolism	24	158	PAICS
hsa05200: Pathways in cancer	24	322	STAT1 STAT3
hsa04020: Calcium signaling pathway	24	173	ADRB2
hsa04144: Endocytosis	24	194	ADRB2
hsa04080: Neuroactive ligand-receptor interaction	24	269	ADRB2
hsa01100: Metabolic pathways	24	1083	PAICS AHCY PCK1

In the table 4.8 functional annotation were done which shows no of different overlapping genes, in the process of Antigen processing and presentation mostly overlapping genes were retrieved; in Graft-versus-host disease the two genes KIR3DS1, KIR3DL2 were identified and in Metabolic pathways PAICS, AHCY, PCK1 were identified. There were many other genes that shows overlapping in different processes.

4.8 Discussion

The purpose of this study was to determine the association of asthma and with three selected diseases as sinusitis, rhinosinusitis and gastroesophageal reflux. Genes involved in each disease were selected and then screen for their common association with target diseases. All of the common genes retrieved have strong association with asthma and selected diseases [130]. IL12RB2, COX1, IL4R, HLA-A and HLA-B are common genes of asthma and sinusitis [224]. LUNX, TSLP, CD284, ALRH, IL33, HLA-DQ, HLA-A and HLA-B are common genes between asthma and rhinosinusitis [225, 226]. COX-2, CD151, TP53, HLA-A and HLA-B common genes between asthma and gastroesophageal reflux disease show a large level of interconnection related to genes [227]. HLA-A and HLA-B were the most common genes that were present all these diseases. HLA-A and HLA-B is part of the HLA Class I heavy chain paralogues. This class 1 molecule is a heavy chain and light chain heterodimer. The strong chain is fixed to the membrane. In the immune system, class I molecules play an important role by incorporating peptides taken from endoplasmic reticulum lumens to identify cytotoxic T cells. They are expressed in virtually every cell [228]. The heavy chain is around 45 kDa and 8 exones are found in its gene. Exon 1 encodes leading peptide, exon 2 and 3 encodes the alpha1 and alpha2 domains, binding all peptide, exon 4 alpha3 domain, exon 5 transmembrane region and exon 6 and 7 encodes the cytoplasm tail. In exon 2 and exon3, the peptide binding specificity for any molecule of class 1 is responsible for polymorphism. Typing of these polymorphisms is regularly conducted for bone marrow and kidney transplantation. More than 6000 HLA-A alleles have been identified [229, 230].

Multiple pathways have been reported to be involved in these diseases and 19 pathways retrieved from different databases showed common association or involvement with asthma and selected comorbidities thus potentially involved in allergic and non-allergic multimorbidity mechanisms. Five of the pathways selected on the basis of common genes involvement includes Antigen processing and presentation, phagosomes, natural killer cell regulated cytotoxicity, cell adhesion molecules and

endocytosis are selected pathways. These pathways are involved in all selected diseases like Antigen presenting presenting pathway involved in the identification of allergens and immune response infectants in asthma, [231] sinusitis, rhinosinusitis [232] and help in inflammation and tissue remodeling [233].Phagosome pathway responsible to destroy the foreign particles and macrophages stimulate during this pathway in asthma, sinusitis and rhinosinusitis [234, 235], recognize metabolic regulators in gastroesophageal reflux disease [236].Natural killing pathway act cytotoxic against infected cells related to the all diseases [237].Cell adhesion molecule pathway in upregulate the secretory process of eosinophils to many stimuli [238].Endocytosis pathways play role to detect and recognize pharyngeal acidification in gastroesophageal reflux and engulf foreign particle like allergens [239].After that retrieved HLA-A and HLA-B interacting proteins from HIPPIE and Innate DB. From these databases retrieved common 3 proteins of HLA-A and 23 proteins of HLA-B selected for construction of network. Common genes, in HLA-A gene these are KIR3DL2 and KIR3DS1 help B27 bound to KIR3DL1, KIR3DL2 and LILRB2 but not LILRB1. KIR3DL2 ligation by B27 inhibited NK and T cell IFN-gamma production [240], and STAT3 The STAT3 transcription regulator has important roles in the production and mature function of the tissue, including inflammation and immunity regulation [241]. In HLA-B proteins are B2M binds the Class I major-histocompatibility-complex (MHC) molecules alpha1, alpha 2 and alpha-3 domains in a non-covalent manner [242], in CD8A When the molecules in human leucocyte antigen (HLA-I) are complexed with antigenic peptides, the CD8+T cell response can be triggered by interaction with the T cell receptor (TCR) and the CD8 co-receiver [243], LILRB1, LILRB2, EZR, MSN, PL53, PSMD1, PAICS, RUUBLI, AHCY, VCP, TRIM28, ADRB2, ARHGEF4, EDEM1, HSPA5, NEDD8, PCK1, STAT1, SUM01, TRIB3 and UBD proteins are common. Proteins common in interaction with HLA-A and HLA-B will be investigated in further detail for their association to the other non-implicated protein or directly retrieved from fun-coup a list of newly predicted multimorbidity related proteins, a functional network of associations were also created [244]. Finally, crosstalk run between all the common extracted proteins from Innate DB and

HIPPIE and find functional annotations of these genes from David tool [221]. The result of David tool gave us different clusters, we selected 1 cluster for HLA-A and 7 clusters of HLA-B by the enrichment score is $\geq$1 and p value as $\leq$0.1 as threshold. Identify enrichment analysis of both genes (HLA-A and HLA-B) to check the most overlapping genes when performed enrichment analysis there are several clusters that shows the significant value that is < 0.05. Huang, and Sherman, highlighted the significant role of related to HLA-A and HLA-B [245]. In HLA-A annotation cluster significant value indicate that these proteins are not clustered accidentally rather they are significantly more enriched. Most probably 4 genes were involved, the functions of these genes are involved in immunoglobin domain, plasma membrane, integral component of plasma membrane, disulphide bond signal peptide, topological domain(extracellular), glycosylation site, glycoprotein, transmembrane region, polymorphism, sequence variant and alternative splicing. In HLA-B functional annotation 6 clusters are significant (cluster 1, 2, 3, 4, 5 and 7) The functions of these genes are involved in MHC class 1 protein binding, regulation of immune response, immunity, cellular response to lipopolysaccharide, Immunoglobulin-like domain, adaptive immunity, immune response, antigen processing and presentation, plasma membrane, external side of plasma membrane, cell surface receptor signaling pathway and many other functions associated to them. And then molecular annotation were done which shows no of different overlapping genes, in the process of Antigen processing and presentation mostly overlapping genes were retrieved; in Graft-versus-host disease the two genes KIR3DS1, KIR3DL2 were identified and in Metabolic pathways PAICS, AHCY, PCK1 were identified. There were many other genes that shows overlapping in different processes [222].

Chapter 5

Conclusion

5.1 Conclusions and Recommendations

Asthma has reported to be associated with many different diseases CRS, rhinitis, SARS, sinusitis, rhinosinusitis and gastroesophageal reflux diseases. Sinusitis, rhinosinusitis and asthma are categorized as allergic diseases whereas gastroesophageal reflux disease is a non-allergic disease but still it is being reported to be as comorbidities with asthma. Current study also revealed these allergic and non-allergic diseases associated as comorbidity, HLA-A and HLA-B that are involved in antigen presentation and a part of immune system. They have strong interaction with many proteins and are involved in many pathways. Reterival of common proteins involved in the targeted disease also shows their interaction with these two (HLA-A and HLA-B) screened genes. Functional annotation results also shows that shared common genes that have significant role in their multimorbidity association.

The outcome of current study needs to be a validated in in-vitro studies. Moreover the common proteins predicted must be evaluated as drug target.

Bibliography

[1] A. Johansson, E. Millqvist, S. Nordin, and M. Bende, "Relationship Between Self-Reported Odor Intolerance and Sensitivity to Inhaled Capsaicin," *Chest*, vol. 129, no. 6, pp. 1623–1628, Jun. 2006. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0012369215507691

[2] N. Lind, M. Nordin, E. Palmquist, A.-S. Claeson, E. Millqvist, and S. Nordin, "Coping and social support in asthma and allergy: the Västerbotten Environmental Health Study," *Journal of Asthma*, vol. 52, no. 6, pp. 622–629, Jul. 2015. [Online]. Available: https://www.tandfonline.com/doi/full/10.3109/02770903.2014.991970

[3] H. K. Reddel, D. R. Taylor, E. D. Bateman, L.-P. Boulet, H. A. Boushey, W. W. Busse, T. B. Casale, P. Chanez, P. L. Enright, P. G. Gibson, J. C. de Jongste, H. A. M. Kerstjens, S. C. Lazarus, M. L. Levy, P. M. O'Byrne, M. R. Partridge, I. D. Pavord, M. R. Sears, P. J. Sterk, S. W. Stoloff, S. D. Sullivan, S. J. Szefler, M. D. Thomas, and S. E. Wenzel, "An Official American Thoracic Society/European Respiratory Society Statement: Asthma Control and Exacerbations: Standardizing Endpoints for Clinical Asthma Trials and Clinical Practice," *American Journal of Respiratory and Critical Care Medicine*, vol. 180, no. 1, pp. 59–99, Jul. 2009. [Online]. Available: http://www.atsjournals.org/doi/abs/10.1164/rccm.200801-060ST

[4] J. Lötvall, L. Ekerljung, E. P. Rönmark, G. Wennergren, A. Lindén, E. Rönmark, K. Torén, and B. Lundbäck, "West Sweden Asthma Study: Prevalence trends over the last 18 years argues no recent increase in

asthma," *Respiratory Research*, vol. 10, no. 1, p. 94, Dec. 2009. [Online]. Available: http://respiratory-research.biomedcentral.com/articles/10.1186/1465-9921-10-94

[5] E. R. Sutherland and W. W. Busse, "Designing clinical trials to address the needs of childhood and adult asthma: The National Heart, Lung, and Blood Institute's AsthmaNet," *Journal of Allergy and Clinical Immunology*, vol. 133, no. 1, pp. 34–38.e1, Jan. 2014. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0091674913016382

[6] J. Chen, L. Liu, Y.-C. T. Shih, D. Zhang, and T. A. Severini, "A flexible model for correlated medical costs, with application to medical expenditure panel survey data: J. CHEN *ET AL.*" *Statistics in Medicine*, vol. 35, no. 6, pp. 883–894, Mar. 2016. [Online]. Available: http://doi.wiley.com/10.1002/sim.6743

[7] R. J. Barrios, F. Kheradmand, L. Batts, and D. B. Corry, "Asthma: pathology and pathophysiology," *Archives of Pathology & Laboratory Medicine*, vol. 130, no. 4, pp. 447–451, Apr. 2006.

[8] M. A. Rank, J. T. Liesinger, M. E. Branda, M. R. Gionfriddo, M. Schatz, R. S. Zeiger, and N. D. Shah, "Comparative safety and costs of stepping down asthma medications in patients with controlled asthma," *Journal of Allergy and Clinical Immunology*, vol. 137, no. 5, pp. 1373–1379.e3, May 2016. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0091674915012609

[9] B. Gesundheit, "Maimonides' appreciation for medicine," *Rambam Maimonides Medical Journal*, vol. 2, no. 1, Jan. 2011. [Online]. Available: https://www.rmmj.org.il/issues/3/Articles/31

[10] J. H. Daruna, *Introduction to psychoneuroimmunology*, second edition ed. Amsterdam ; Boston: Elsevier, AP, 2012.

[11] A. Homei, "Michael Shiyung Liu, Prescribing Colonization: The Role of Medical Practices and Policies in Japan-Ruled Taiwan, 1895-1945,"

Social History of Medicine, vol. 24, no. 3, pp. 858–859, Dec. 2011. [Online]. Available: https://academic.oup.com/shm/article-lookup/doi/10.1093/shm/hkr121

[12] S. Kojima, M. Yokogawa, and T. Tada, "Production and properties of reaginic antibodies in rabbits infected with Clonorchis sinensis or Schistosoma japonicum," *Experimental Parasitology*, vol. 35, no. 1, pp. 141–149, Feb. 1974. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/0014489474900174

[13] M. J. Walter and M. J. Holtzman, "A Centennial History of Research on Asthma Pathogenesis," *American Journal of Respiratory Cell and Molecular Biology*, vol. 32, no. 6, pp. 483–489, Jun. 2005. [Online]. Available: http://www.atsjournals.org/doi/abs/10.1165/rcmb.F300

[14] J. Douwes, C. Brooks, C. van Dalen, and N. Pearce, "Importance of Allergy in Asthma: An Epidemiologic Perspective," *Current Allergy and Asthma Reports*, vol. 11, no. 5, pp. 434–444, Oct. 2011. [Online]. Available: http://link.springer.com/10.1007/s11882-011-0215-6

[15] M. Perpiñá Tordera, "La complejidad en el asma: inflamación y redes libres de escala," *Archivos de Bronconeumología*, vol. 45, no. 9, pp. 459–465, Sep. 2009. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0300289609002476

[16] E. Harada, S. Moriya, A. Murata, M. Muramatsu, H. Kashiwazaki, K. Kobayashi, K. Notani, and N. Inoue, "Relationship between subjective assessment of oral health and medical expenses in community-dwelling elderly persons," *Gerodontology*, vol. 29, no. 2, pp. e246–252, Jun. 2012.

[17] S. T. Holgate, "Pathophysiology of asthma: What has our current understanding taught us about new therapeutic approaches?" *Journal of Allergy and Clinical Immunology*, vol. 128, no. 3, pp. 495–505, Sep. 2011. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S009167491101092X

[18] P. Subbarao, P. J. Mandhane, and M. R. Sears, "Asthma: epidemiology, etiology and risk factors," *Canadian Medical Association Journal*, vol. 181, no. 9, pp. E181–E190, Oct. 2009. [Online]. Available: http://www.cmaj.ca/cgi/doi/10.1503/cmaj.080612

[19] V. Garcia-Larsen, J. F. Potts, E. Omenaas, J. Heinrich, C. Svanes, J. Garcia-Aymerich, P. G. Burney, and D. Jarvis, "Dietary antioxidants and 10-year lung function decline in adults from the ECRHS survey," *European Respiratory Journal*, vol. 50, no. 6, p. 1602286, Dec. 2017. [Online]. Available: http://erj.ersjournals.com/lookup/doi/10.1183/13993003.02286-2016

[20] E. D. Bateman, S. S. Hurd, P. J. Barnes, J. Bousquet, J. M. Drazen, M. FitzGerald, P. Gibson, K. Ohta, P. O'Byrne, S. E. Pedersen, E. Pizzichini, S. D. Sullivan, S. E. Wenzel, and H. J. Zar, "Global strategy for asthma management and prevention: GINA executive summary," *European Respiratory Journal*, vol. 31, no. 1, pp. 143–178, Jan. 2008. [Online]. Available: http://erj.ersjournals.com/cgi/doi/10.1183/09031936.00138707

[21] B. Leynaert, C. Neukirch, R. Liard, J. Bousquet, and F. Neukirch, "Quality of Life in Allergic Rhinitis and Asthma: A Population-based Study of Young Adults," *American Journal of Respiratory and Critical Care Medicine*, vol. 162, no. 4, pp. 1391–1396, Oct. 2000. [Online]. Available: http://www.atsjournals.org/doi/abs/10.1164/ajrccm.162.4.9912033

[22] Y. Chida, M. Hamer, and A. Steptoe, "A Bidirectional Relationship Between Psychosocial Factors and Atopic Disorders: A Systematic Review and Meta-Analysis:," *Psychosomatic Medicine*, vol. 70, no. 1, pp. 102–116, Jan. 2008. [Online]. Available: http://journals.lww.com/00006842-200801000-00015

[23] L. Wang, X. Shen, Y. Wu, and D. Zhang, "Coffee and caffeine consumption and depression: A meta-analysis of observational studies," *Australian & New Zealand Journal of Psychiatry*, vol. 50, no. 3, pp. 228–242, Mar. 2016. [Online]. Available: http://journals.sagepub.com/doi/10.1177/0004867415603131

[24] M. Niedoszytko, M. Gruchała-Niedoszytko, and E. Jassem, "Gene expression analysis in allergology: the prediction of Hymenoptera venom allergy severity and treatment efficacy," *Clinical and Translational Allergy*, vol. 3, no. 1, p. 35, Dec. 2013. [Online]. Available: https://ctajournal.biomedcentral.com/articles/10.1186/2045-7022-3-35

[25] J. Hellgren, A. Cervin, S. Nordling, A. Bergman, and L. O. Cardell, "Allergic rhinitis and the common cold - high cost to society: The burden of rhinitis," *Allergy*, vol. 65, no. 6, pp. 776–783, Nov. 2009. [Online]. Available: http://doi.wiley.com/10.1111/j.1398-9995.2009.02269.x

[26] S. Micheal, K. Minhas, M. Ishaque, F. Ahmed, and A. Ahmed, "Promoter polymorphisms of the CD14 gene are associated with atopy in Pakistani adults," *Journal of Investigational Allergology & Clinical Immunology*, vol. 21, no. 5, pp. 394–397, 2011.

[27] M. Shazia, M. Kanza, I. Mehwish, S. Irum, A. Farida, and A. Asifa, "IL-13 gene polymorphisms and their association with atopic asthma and rhinitis in Pakistani patients," *Iranian Journal of Allergy, Asthma, and Immunology*, vol. 12, no. 4, pp. 391–396, Aug. 2013.

[28] H. Li, Y. Li, M. Zhang, G. Xu, X. Feng, J. Xi, and B. Zhao, "Associations of genetic variants in ADAM33 and TGF-β1 genes with childhood asthma risk," *Biomedical Reports*, vol. 2, no. 4, pp. 533–538, Jul. 2014. [Online]. Available: https://www.spandidos-publications.com/10.3892/br.2014.280

[29] A. El-Dib, H. Nagy, W. Sabry, and W. El-Shimy, "A study of IL-6, IL-8, and TNF-α as inflammatory markers in COPD patients," *Egyptian Journal of Bronchology*, vol. 8, no. 2, p. 91, 2014. [Online]. Available: http://www.ejbronchology.eg.net/text.asp?2014/8/2/91/145698

[30] F. Bottacini, D. van Sinderen, and M. Ventura, "Omics of bifidobacteria: research and insights into their health-promoting activities," *Biochemical Journal*, vol. 474, no. 24, pp. 4137–4152, Dec. 2017.

[Online]. Available: https://portlandpress.com/biochemj/article/474/24/4137/49629/Omics-of-bifidobacteria-research-and-insights-into

[31] E. G. Weinberg, "Urbanization and childhood asthma: An African perspective," *Journal of Allergy and Clinical Immunology*, vol. 105, no. 2, pp. 224–231, Feb. 2000. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0091674900900691

[32] Y. Huo and H.-Y. Zhang, "Genetic Mechanisms of Asthma and the Implications for Drug Repositioning," *Genes*, vol. 9, no. 5, p. 237, May 2018. [Online]. Available: http://www.mdpi.com/2073-4425/9/5/237

[33] N. Taniguchi, S. Konno, T. Hattori, A. Isada, K. Shimizu, K. Shimizu, N. Shijubo, S.-K. Huang, N. Hizawa, and M. Nishimura, "The CC16 A38G polymorphism is associated with asymptomatic airway hyper-responsiveness and development of late-onset asthma," *Annals of Allergy, Asthma & Immunology*, vol. 111, no. 5, pp. 376–381.e1, Nov. 2013. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S1081120613005723

[34] B. A. Raby, K. Van Steen, R. Lazarus, J. C. Celedón, E. K. Silverman, and S. T. Weiss, "Eotaxin polymorphisms and serum total IgE levels in children with asthma," *Journal of Allergy and Clinical Immunology*, vol. 117, no. 2, pp. 298–305, Feb. 2006. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S009167490502508X

[35] Y.-G. Zhang, J. Huang, J. Zhang, X.-B. Li, C. He, Y.-L. Xiao, C. Tian, H. Wan, Y.-L. Zhao, Y.-G. Tsewang, and H. Fan, "RANTES Gene Polymorphisms and Asthma Risk: A Meta-Analysis," *Archives of Medical Research*, vol. 41, no. 1, pp. 50–58, Jan. 2010. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0188440910000032

[36] S. Moncini, M. T. Bonati, I. Morella, L. Ferrari, R. Brambilla, and P. Riva, "Differential allelic expression of SOS1 and hyperexpression of the activating SOS1 c.755C variant in a Noonan syndrome family," *European*

Journal of Human Genetics, vol. 23, no. 11, pp. 1531–1537, Oct. 2015. [Online]. Available: http://www.nature.com/articles/ejhg201520

[37] G. Silva, F. Naveca, R. Ramasawmy, and A. Boechat, "Association between the IFNG +874A/T gene polymorphism and leprosy resistance: A meta-analysis," *Cytokine*, vol. 65, no. 2, pp. 130–133, Feb. 2014. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S1043466613007643

[38] M.-H. Hyun, C.-H. Lee, M.-H. Kang, B.-K. Park, and Y. H. Lee, "Interleukin-10 Promoter Gene Polymorphisms and Susceptibility to Asthma: A Meta-Analysis," *PLoS ONE*, vol. 8, no. 1, p. e53758, Jan. 2013. [Online]. Available: https://dx.plos.org/10.1371/journal.pone.0053758

[39] J. Padrón-Morales, C. Sanz, I. Dávila, F. Muñoz-Bellido, F. Lorente, and M. Isidoro-García, "Polymorphisms of the IL12B, IL1B, and TNFA genes and susceptibility to asthma," *Journal of Investigational Allergology & Clinical Immunology*, vol. 23, no. 7, pp. 487–494, 2013.

[40] S. Accordini, L. Calciano, C. Bombieri, G. Malerba, F. Belpinati, A. R. Lo Presti, A. Baldan, M. Ferrari, L. Perbellini, and R. de Marco, "An Interleukin 13 Polymorphism Is Associated with Symptom Severity in Adult Subjects with Ever Asthma," *PLOS ONE*, vol. 11, no. 3, p. e0151292, Mar. 2016. [Online]. Available: https://dx.plos.org/10.1371/journal.pone.0151292

[41] M. F. Moffatt, I. G. Gut, F. Demenais, D. P. Strachan, E. Bouzigon, S. Heath, E. von Mutius, M. Farrall, M. Lathrop, and W. O. Cookson, "A Large-Scale, Consortium-Based Genomewide Association Study of Asthma," *New England Journal of Medicine*, vol. 363, no. 13, pp. 1211–1221, Sep. 2010. [Online]. Available: http://www.nejm.org/doi/abs/10.1056/NEJMoa0906312

[42] O. E. Savenije, J. M. Mahachie John, R. Granell, M. Kerkhof, F. N. Dijk, J. C. de Jongste, H. A. Smit, B. Brunekreef, D. S. Postma, K. Van Steen, J. Henderson, and G. H. Koppelman, "Association of IL33–IL-1 receptor–like 1 (IL1RL1) pathway polymorphisms with wheezing

phenotypes and asthma in childhood," *Journal of Allergy and Clinical Immunology*, vol. 134, no. 1, pp. 170–177, Jul. 2014. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0091674914000591

[43] L. Tang, H. Lin, and B. Chen, "Association of IL-4 promoter polymorphisms with asthma: a meta-analysis," *Genetics and Molecular Research*, vol. 13, no. 1, pp. 1383–1394, 2014. [Online]. Available: http://www.funpecrp.com.br/gmr/year2014/vol13-1/pdf/gmr2976.pdf

[44] A. A. Amirzargar, M. Movahedi, N. Rezaei, B. Moradi, S. Dorkhosh, M. Mahloji, and S. A. Mahdaviani, "Polymorphisms in IL4 and iLARA confer susceptibility to asthma," *Journal of Investigational Allergology & Clinical Immunology*, vol. 19, no. 6, pp. 433–438, 2009.

[45] M. B. Freidin, O. S. Kobyakova, L. M. Ogorodova, and V. P. Puzyrev, "Association of Polymorphisms in the Human *IL4* and *IL5* Genes with Atopic Bronchial Asthma and Severity of the Disease," *Comparative and Functional Genomics*, vol. 4, no. 3, pp. 346–350, 2003. [Online]. Available: http://www.hindawi.com/journals/ijg/2003/743029/abs/

[46] E. Babusikova, J. Jurecekova, M. Jesenak, and A. Evinova, "Asociación entre polimorfismos genéticos de la interleucina 6 y el asma bronquial en niños," *Archivos de Bronconeumología*, vol. 53, no. 7, pp. 381–386, Jul. 2017. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0300289616302551

[47] J. s. Kim, J. Choi, H.-J. Hahn, Y.-B. Lee, D.-S. Yu, and J.-W. Kim, "Association of Macrophage Migration Inhibitory Factor Polymorphisms with Total Plasma IgE Levels in Patients with Atopic Dermatitis in Korea," *PLOS ONE*, vol. 11, no. 9, p. e0162477, Sep. 2016. [Online]. Available: https://dx.plos.org/10.1371/journal.pone.0162477

[48] X. Qian, Y. Gao, X. Ye, and M. Lu, "Association of STAT6 variants with asthma risk: A systematic review and meta-analysis," *Human*

Immunology, vol. 75, no. 8, pp. 847–853, Aug. 2014. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0198885914001712

[49] M. Harada, T. Hirota, A. I. Jodo, Y. Hitomi, M. Sakashita, T. Tsunoda, T. Miyagawa, S. Doi, M. Kameda, K. Fujita, A. Miyatake, T. Enomoto, E. Noguchi, H. Masuko, T. Sakamoto, N. Hizawa, Y. Suzuki, S. Yoshihara, M. Adachi, M. Ebisawa, H. Saito, K. Matsumoto, T. Nakajima, R. A. Mathias, N. Rafaels, K. C. Barnes, B. E. Himes, Q. L. Duan, K. G. Tantisira, S. T. Weiss, Y. Nakamura, S. F. Ziegler, and M. Tamari, "Thymic Stromal Lymphopoietin Gene Promoter Polymorphisms Are Associated with Susceptibility to Bronchial Asthma," *American Journal of Respiratory Cell and Molecular Biology*, vol. 44, no. 6, pp. 787–793, Jun. 2011. [Online]. Available: http://www.atsjournals.org/doi/abs/10.1165/rcmb.2009-0418OC

[50] M. Crowhurst, "Teaching and Learning the Writing of Persuasive/Argumentative Discourse," *Canadian Journal of Education / Revue canadienne de l'éducation*, vol. 15, no. 4, p. 348, 1990. [Online]. Available: https://www.jstor.org/stable/1495109?origin=crossref

[51] M. Schatz and C. A. Camargo, "The relationship of sex to asthma prevalence, health care utilization, and medications in a large managed care organization," *Annals of Allergy, Asthma & Immunology*, vol. 91, no. 6, pp. 553–558, Dec. 2003. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S1081120610615335

[52] Y. Bossé and T. J. Hudson, "Toward a Comprehensive Set of Asthma Susceptibility Genes," *Annual Review of Medicine*, vol. 58, no. 1, pp. 171–184, Feb. 2007. [Online]. Available: http://www.annualreviews.org/doi/10.1146/annurev.med.58.071105.111738

[53] D. Vercelli, "Discovering susceptibility genes for asthma and allergy," *Nature Reviews Immunology*, vol. 8, no. 3, pp. 169–182, Mar. 2008. [Online]. Available: http://www.nature.com/articles/nri2257

[54] D. B. Peden, "Development of atopy and asthma: candidate environmental influences and important periods of exposure." *Environmental Health Perspectives*, vol. 108, no. suppl 3, pp. 475–482, Jun. 2000. [Online]. Available: https://ehp.niehs.nih.gov/doi/10.1289/ehp.00108s3475

[55] G. B. Diette, M. C. McCormack, N. N. Hansel, P. N. Breysse, and E. C. Matsui, "Environmental issues in managing asthma," *Respiratory Care*, vol. 53, no. 5, pp. 602–615; discussion 616–617, May 2008.

[56] S. Ghosh, P. Pahwa, D. C. Rennie, and B. Janzen, "Gender-related interactive effect of smoking and rural/urban living on asthma prevalence: a longitudinal Canadian NPHS study," *The Journal of Asthma: Official Journal of the Association for the Care of Asthma*, vol. 46, no. 10, pp. 988–994, Dec. 2009.

[57] S. F. Thomsen, D. L. Duffy, K. O. Kyvik, and V. Backer, "Genetic influence on the age at onset of asthma: a twin study," *The Journal of Allergy and Clinical Immunology*, vol. 126, no. 3, pp. 626–630, Sep. 2010.

[58] L. Avila, M. E. Soto-Martínez, M. E. Soto-Quirós, and J. C. Celedón, "Asthma, current wheezing, and tobacco use among adolescents and young adults in Costa Rica," *The Journal of Asthma: Official Journal of the Association for the Care of Asthma*, vol. 42, no. 7, pp. 543–547, Sep. 2005.

[59] F. Laden, Y.-H. Chiu, E. Garshick, S. K. Hammond, and J. E. Hart, "A cross-sectional study of secondhand smoke exposure and respiratory symptoms in non-current smokers in the U.S. trucking industry: SHS exposure and respiratory symptoms," *BMC public health*, vol. 13, p. 93, Feb. 2013.

[60] ——, "A cross-sectional study of secondhand smoke exposure and respiratory symptoms in non-current smokers in the U.S. trucking industry: SHS exposure and respiratory symptoms," *BMC public health*, vol. 13, p. 93, Feb. 2013.

[61] E. D. Bateman, S. S. Hurd, P. J. Barnes, J. Bousquet, J. M. Drazen, J. M. FitzGerald, P. Gibson, K. Ohta, P. O'Byrne, S. E. Pedersen, E. Pizzichini,

S. D. Sullivan, S. E. Wenzel, and H. J. Zar, "Global strategy for asthma management and prevention: GINA executive summary," *The European Respiratory Journal*, vol. 31, no. 1, pp. 143–178, Jan. 2008.

[62] L. T. Stiemsma, L. A. Reynolds, S. E. Turvey, and B. B. Finlay, "The hygiene hypothesis: current perspectives and future therapies," *ImmunoTargets and Therapy*, vol. 4, pp. 143–157, 2015.

[63] M. K. Vernon, J. A. Bell, I. Wiklund, P. Dale, and K. R. Chapman, "Asthma Control and Asthma Triggers: The Patient Perspective," *Journal of Asthma & Allergy Educators*, vol. 4, no. 4, pp. 155–164, Aug. 2013. [Online]. Available: http://journals.sagepub.com/doi/10.1177/2150129713483307

[64] A. Papi, F. Blasi, G. W. Canonica, L. Morandi, L. Richeldi, and A. Rossi, "Treatment strategies for asthma: reshaping the concept of asthma management," *Allergy, Asthma & Clinical Immunology*, vol. 16, no. 1, p. 75, Dec. 2020. [Online]. Available: https://aacijournal.biomedcentral.com/articles/10.1186/s13223-020-00472-8

[65] S. C. Hall and D. K. Agrawal, "Vitamin D and Bronchial Asthma: An Overview of Data From the Past 5 Years," *Clinical Therapeutics*, vol. 39, no. 5, pp. 917–929, May 2017.

[66] M. Yurt, J. Liu, R. Sakurai, M. Gong, S. M. Husain, M. A. Siddiqui, M. Husain, P. Villarreal, F. Akcay, J. S. Torday, and V. K. Rehan, "Vitamin D supplementation blocks pulmonary structural and functional changes in a rat model of perinatal vitamin D deficiency," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 307, no. 11, pp. L859–867, Dec. 2014.

[67] A. Bashir and A. A. Litonjua, "Observational studies of vitamin D associations with asthma: Problems and pitfalls," *Pediatric Pulmonology*, vol. 53, no. 10, pp. 1338–1339, Oct. 2018.

[68] H. M. Wolsk, B. L. Chawes, A. A. Litonjua, B. W. Hollis, J. Waage, J. Stokholm, K. Bønnelykke, H. Bisgaard, and S. T. Weiss, "Prenatal vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood: A combined analysis of two randomized controlled trials," *PloS One*, vol. 12, no. 10, p. e0186657, 2017.

[69] D. A. Jolliffe, L. Greenberg, R. L. Hooper, C. J. Griffiths, C. A. Camargo, C. P. Kerley, M. E. Jensen, D. Mauger, I. Stelmach, M. Urashima, and A. R. Martineau, "Vitamin D supplementation to prevent asthma exacerbations: a systematic review and meta-analysis of individual participant data," *The Lancet. Respiratory Medicine*, vol. 5, no. 11, pp. 881–890, Nov. 2017.

[70] M. Castro, T. S. King, S. J. Kunselman, M. D. Cabana, L. Denlinger, F. Holguin, S. D. Kazani, W. C. Moore, J. Moy, C. A. Sorkness, P. Avila, L. B. Bacharier, E. Bleecker, H. A. Boushey, J. Chmiel, A. M. Fitzpatrick, D. Gentile, M. Hundal, E. Israel, M. Kraft, J. A. Krishnan, C. LaForce, S. C. Lazarus, R. Lemanske, N. Lugogo, R. J. Martin, D. T. Mauger, E. Naureckas, S. P. Peters, W. Phipatanakul, L. G. Que, A. Sheshadri, L. Smith, J. Solway, L. Sullivan-Vedder, K. Sumino, M. E. Wechsler, S. Wenzel, S. R. White, E. R. Sutherland, and National Heart, Lung, and Blood Institute's AsthmaNet, "Effect of vitamin D3 on asthma treatment failures in adults with symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial," *JAMA*, vol. 311, no. 20, pp. 2083–2091, May 2014.

[71] D. Heederik and E. von Mutius, "Does diversity of environmental microbial exposure matter for the occurrence of allergy and asthma?" *The Journal of Allergy and Clinical Immunology*, vol. 130, no. 1, pp. 44–50, Jul. 2012.

[72] T. Olszak, D. An, S. Zeissig, M. P. Vera, J. Richter, A. Franke, J. N. Glickman, R. Siebert, R. M. Baron, D. L. Kasper, and R. S. Blumberg, "Microbial exposure during early life has persistent effects on natural killer T cell function," *Science (New York, N.Y.)*, vol. 336, no. 6080, pp. 489–493, Apr. 2012.

[73] W. Eder, M. J. Ege, and E. von Mutius, "The Asthma Epidemic," *New England Journal of Medicine*, vol. 355, no. 21, pp. 2226–2235, Nov. 2006. [Online]. Available: http://www.nejm.org/doi/abs/10.1056/NEJMra054308

[74] M. Waser, K. B. Michels, C. Bieli, H. Flöistrup, G. Pershagen, E. von Mutius, M. Ege, J. Riedler, D. Schram-Bijkerk, B. Brunekreef, M. van Hage, R. Lauener, C. Braun-Fahrländer, and PARSIFAL Study team, "Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe," *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology*, vol. 37, no. 5, pp. 661–670, May 2007.

[75] Y. J. Huang and H. A. Boushey, "The microbiome in asthma," *The Journal of Allergy and Clinical Immunology*, vol. 135, no. 1, pp. 25–30, Jan. 2015.

[76] M. B. Azad, T. Konya, R. R. Persaud, D. S. Guttman, R. S. Chari, C. J. Field, M. R. Sears, P. J. Mandhane, S. E. Turvey, P. Subbarao, A. B. Becker, J. A. Scott, A. L. Kozyrskyj, and CHILD Study Investigators, "Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study," *BJOG: an international journal of obstetrics and gynaecology*, vol. 123, no. 6, pp. 983–993, May 2016.

[77] T. P. Wypych and B. J. Marsland, "Antibiotics as Instigators of Microbial Dysbiosis: Implications for Asthma and Allergy," *Trends in Immunology*, vol. 39, no. 9, pp. 697–711, Sep. 2018.

[78] J. E. Sordillo, Y. Zhou, M. J. McGeachie, J. Ziniti, N. Lange, N. Laranjo, J. R. Savage, V. Carey, G. O'Connor, M. Sandel, R. Strunk, L. Bacharier, R. Zeiger, S. T. Weiss, G. Weinstock, D. R. Gold, and A. A. Litonjua, "Factors influencing the infant gut microbiome at age 3-6 months: Findings from the ethnically diverse Vitamin D Antenatal Asthma Reduction Trial (VDAART)," *The Journal of Allergy and Clinical Immunology*, vol. 139, no. 2, pp. 482–491.e14, Feb. 2017.

[79] N. T. Ho, F. Li, K. A. Lee-Sarwar, H. M. Tun, B. P. Brown, P. S. Pannaraj, J. M. Bender, M. B. Azad, A. L. Thompson, S. T. Weiss, M. A. Azcarate-Peril, A. A. Litonjua, A. L. Kozyrskyj, H. B. Jaspan, G. M. Aldrovandi, and L. Kuhn, "Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations," *Nature Communications*, vol. 9, no. 1, p. 4169, Oct. 2018.

[80] M. Harju, L. Keski-Nisula, L. Georgiadis, and S. Heinonen, "Parental smoking and cessation during pregnancy and the risk of childhood asthma," *BMC public health*, vol. 16, p. 428, May 2016.

[81] C. T. McEvoy and E. R. Spindel, "Pulmonary Effects of Maternal Smoking on the Fetus and Child: Effects on Lung Development, Respiratory Morbidities, and Life Long Lung Health," *Paediatric Respiratory Reviews*, vol. 21, pp. 27–33, Jan. 2017.

[82] K. Gibbs, J. M. Collaco, and S. A. McGrath-Morrow, "Impact of Tobacco Smoke and Nicotine Exposure on Lung Development," *Chest*, vol. 149, no. 2, pp. 552–561, Feb. 2016.

[83] H. Burke, J. Leonardi-Bee, A. Hashim, H. Pine-Abata, Y. Chen, D. G. Cook, J. R. Britton, and T. M. McKeever, "Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis," *Pediatrics*, vol. 129, no. 4, pp. 735–744, Apr. 2012.

[84] A. Gref, S. K. Merid, O. Gruzieva, S. Ballereau, A. Becker, T. Bellander, A. Bergström, Y. Bossé, M. Bottai, M. Chan-Yeung, E. Fuertes, D. Ierodiakonou, R. Jiang, S. Joly, M. Jones, M. S. Kobor, M. Korek, A. L. Kozyrskyj, A. Kumar, N. Lemonnier, E. MacIntyre, C. Ménard, D. Nickle, M. Obeidat, J. Pellet, M. Standl, A. Sääf, C. Söderhäll, C. M. T. Tiesler, M. van den Berge, J. M. Vonk, H. Vora, C.-J. Xu, J. M. Antó, C. Auffray, M. Brauer, J. Bousquet, B. Brunekreef, W. J. Gauderman, J. Heinrich, J. Kere, G. H. Koppelman, D. Postma, C. Carlsten, G. Pershagen, and E. Melén, "Genome-Wide Interaction Analysis of Air Pollution Exposure and Childhood Asthma

with Functional Follow-up," *American Journal of Respiratory and Critical Care Medicine*, vol. 195, no. 10, pp. 1373–1383, May 2017.

[85] P. Paggiaro and B. Vagaggini, "[Clinical aspects of chronic obstructive pulmonary disease]," *Annali dell'Istituto Superiore Di Sanita*, vol. 39, no. 4, pp. 519–528, 2003.

[86] S. L. Lee, H. G. Wasserlauf, J. S. Brault, K. R. Freydl, W. A. Earman, and T. O. Glonek, "31P nuclear magnetic resonance spectroscopic evidence that canine nucleus pulposus and anulus fibrosus cells differ in principal organophosphorus biomolecules," *Spine*, vol. 20, no. 21, pp. 2278–2282, Nov. 1995.

[87] S. A. G. Willis-Owen, W. O. C. Cookson, and M. F. Moffatt, "The Genetics and Genomics of Asthma," *Annual Review of Genomics and Human Genetics*, vol. 19, pp. 223–246, Aug. 2018.

[88] V. Ullemar, P. K. E. Magnusson, C. Lundholm, A. Zettergren, E. Melén, P. Lichtenstein, and C. Almqvist, "Heritability and confirmation of genetic association studies for childhood asthma in twins," *Allergy*, vol. 71, no. 2, pp. 230–238, Feb. 2016.

[89] A. Buniello, J. A. L. MacArthur, M. Cerezo, L. W. Harris, J. Hayhurst, C. Malangone, A. McMahon, J. Morales, E. Mountjoy, E. Sollis, D. Suveges, O. Vrousgou, P. L. Whetzel, R. Amode, J. A. Guillen, H. S. Riat, S. J. Trevanion, P. Hall, H. Junkins, P. Flicek, T. Burdett, L. A. Hindorff, F. Cunningham, and H. Parkinson, "The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019," *Nucleic Acids Research*, vol. 47, no. D1, pp. D1005–D1012, Jan. 2019.

[90] B. A. Raby, "Asthma severity, nature or nurture: genetic determinants," *Current Opinion in Pediatrics*, vol. 31, no. 3, pp. 340–348, Jun. 2019.

[91] B. James, S. Milstien, and S. Spiegel, "ORMDL3 and allergic asthma: From physiology to pathology," *The Journal of Allergy and Clinical Immunology*, vol. 144, pp. 634–640, Sep. 2019.

[92] M. F. Moffatt, M. Kabesch, L. Liang, A. L. Dixon, D. Strachan, S. Heath, M. Depner, A. von Berg, A. Bufe, E. Rietschel, A. Heinzmann, B. Simma, T. Frischer, S. A. G. Willis-Owen, K. C. C. Wong, T. Illig, C. Vogelberg, S. K. Weiland, E. von Mutius, G. R. Abecasis, M. Farrall, I. G. Gut, G. M. Lathrop, and W. O. C. Cookson, "Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma," *Nature*, vol. 448, no. 7152, pp. 470–473, Jul. 2007.

[93] R. G. Lima, A. C. Pastorino, R. R. D. Casagrande, D. Sole, C. Leone, and C. M. A. Jacob, "Prevalence of asthma, rhinitis and eczema in 6 - 7 years old students from the western districts of São Paulo City, using the standardized questionnaire of the "International Study of Asthma and Allergies in Childhood" (ISAAC)-phase IIIB," *Clinics (Sao Paulo, Brazil)*, vol. 62, no. 3, pp. 225–234, Jun. 2007.

[94] S. Pakhale, K. Wooldrage, J. Manfreda, and N. Anthonisen, "Prevalence of asthma symptoms in 7th- and 8th-grade school children in a rural region in India," *The Journal of Asthma: Official Journal of the Association for the Care of Asthma*, vol. 45, no. 2, pp. 117–122, Mar. 2008.

[95] G. Mustafa, P. A. Khan, and I. Iqbal, "Nocturnal asthma in school children of south Punjab, Pakistan," *Journal of Ayub Medical College, Abbottabad: JAMC*, vol. 20, no. 3, pp. 36–39, Sep. 2008.

[96] T. H. Jafar, N. Chaturvedi, and G. Pappas, "Prevalence of overweight and obesity and their association with hypertension and diabetes mellitus in an Indo-Asian population," *CMAJ: Canadian Medical Association journal = journal de l'Association medicale canadienne*, vol. 175, no. 9, pp. 1071–1077, Oct. 2006.

[97] T. S. Hallstrand, M. E. Fischer, M. M. Wurfel, N. Afari, D. Buchwald, and J. Goldberg, "Genetic pleiotropy between asthma and obesity in a community-based sample of twins," *The Journal of Allergy and Clinical Immunology*, vol. 116, no. 6, pp. 1235–1241, Dec. 2005.

[98] M. Dorrian, "Regulating architecture," *Architectural Research Quarterly*, vol. 16, no. 3, pp. 200–204, Sep. 2012. [Online]. Available: https://www.cambridge.org/core/product/identifier/S1359135513000031/type/journal_article

[99] D. Jootun, G. McGhee, and G. R. Marland, "Reflexivity: promoting rigour in qualitative research," *Nursing Standard (Royal College of Nursing (Great Britain): 1987)*, vol. 23, no. 23, pp. 42–46, Feb. 2009.

[100] J. S. Fontana, "A methodology for critical science in nursing," *ANS. Advances in nursing science*, vol. 27, no. 2, pp. 93–101, Jun. 2004.

[101] K. Panchal and M. Damodaran, "Computation of the Flowfield in the Vicinity of an Electric Vehicle Platform," in *Fluid Mechanics and Fluid Power – Contemporary Research*, A. K. Saha, D. Das, R. Srivastava, P. K. Panigrahi, and K. Muralidhar, Eds. New Delhi: Springer India, 2017, pp. 333–341, series Title: Lecture Notes in Mechanical Engineering. [Online]. Available: http://link.springer.com/10.1007/978-81-322-2743-4_32

[102] D. Eassey, H. K. Reddel, K. Ryan, and L. Smith, "The impact of severe asthma on patients' autonomy: A qualitative study," *Health Expectations: An International Journal of Public Participation in Health Care and Health Policy*, vol. 22, no. 3, pp. 528–536, Jun. 2019.

[103] B. Sarani and V. Gracias, "Safety and efficacy of propofol," *The Journal of Trauma*, vol. 64, no. 1, p. 242, Jan. 2008.

[104] S. Mysore and R. E. Ruffin, "Long-acting β-agonists in asthma management: what is the current status?" *Drugs*, vol. 71, no. 16, pp. 2091–2097, Nov. 2011.

[105] A. E. B. Yassin, A. H. Aodah, S. Al-Suwayeh, and E. I. Taha, "Theophylline colon specific tablets for chronotherapeutic treatment of nocturnal asthma," *Pharmaceutical Development and Technology*, vol. 17, no. 6, pp. 712–718, Dec. 2012.

[106] S. R. Del Giacco, A. Bakirtas, E. Bel, A. Custovic, Z. Diamant, E. Hamelmann, E. Heffler, . Kalayci, S. Saglani, S. Sergejeva, S. Seys, A. Simpson, and L. Bjermer, "Allergy in severe asthma," *Allergy*, vol. 72, no. 2, pp. 207–220, Feb. 2017.

[107] J. Merckx, F. M. Ducharme, C. Martineau, R. Zemek, J. Gravel, D. Chalut, N. Poonai, C. Quach, and Pediatric Emergency Research Canada (PERC) DOORWAY team, "Respiratory Viruses and Treatment Failure in Children With Asthma Exacerbation," *Pediatrics*, vol. 142, no. 1, Jul. 2018.

[108] D. J. Jackson and S. L. Johnston, "The role of viruses in acute exacerbations of asthma," *The Journal of Allergy and Clinical Immunology*, vol. 125, no. 6, pp. 1178–1187; quiz 1188–1189, Jun. 2010.

[109] W. W. Busse, W. J. Morgan, P. J. Gergen, H. E. Mitchell, J. E. Gern, A. H. Liu, R. S. Gruchalla, M. Kattan, S. J. Teach, J. A. Pongracic, J. F. Chmiel, S. F. Steinbach, A. Calatroni, A. Togias, K. M. Thompson, S. J. Szefler, and C. A. Sorkness, "Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children," *The New England Journal of Medicine*, vol. 364, no. 11, pp. 1005–1015, Mar. 2011.

[110] S. J. Teach, P. J. Gergen, S. J. Szefler, H. E. Mitchell, A. Calatroni, J. Wildfire, G. R. Bloomberg, C. M. Kercsmar, A. H. Liu, M. M. Makhija, E. Matsui, W. Morgan, G. O'Connor, and W. W. Busse, "Seasonal risk factors for asthma exacerbations among inner-city children," *The Journal of Allergy and Clinical Immunology*, vol. 135, no. 6, pp. 1465–1473.e5, Jun. 2015.

[111] M. Patel and D. Shaw, "A review of standard pharmacological therapy for adult asthma – Steps 1 to 5," *Chronic Respiratory Disease*, vol. 12, no. 2, pp. 165–176, May 2015.

[112] S. M. Caress and A. C. Steinemann, "Prevalence of multiple chemical sensitivities: a population-based study in the southeastern United States," *American Journal of Public Health*, vol. 94, no. 5, pp. 746–747, May 2004.

[113] P. Henningsen and S. Priebe, "New environmental illnesses: what are their characteristics?" *Psychotherapy and Psychosomatics*, vol. 72, no. 5, pp. 231–234, Oct. 2003.

[114] M. J. E. Andersson, L. Andersson, M. Bende, E. Millqvist, and S. Nordin, "The idiopathic environmental intolerance symptom inventory: development, evaluation, and application," *Journal of Occupational and Environmental Medicine*, vol. 51, no. 7, pp. 838–847, Jul. 2009.

[115] N. D. Berg, A. Linneberg, A. Dirksen, and J. Elberling, "Prevalence of self-reported symptoms and consequences related to inhalation of airborne chemicals in a Danish general population," *International Archives of Occupational and Environmental Health*, vol. 81, no. 7, pp. 881–887, Jul. 2008.

[116] F. Carlsson, B. Karlson, P. Ørbaek, K. Osterberg, and P.-O. Ostergren, "Prevalence of annoyance attributed to electrical equipment and smells in a Swedish population, and relationship with subjective health and daily functioning," *Public Health*, vol. 119, no. 7, pp. 568–577, Jul. 2005.

[117] C. Hausteiner, S. Bornschein, T. Zilker, P. Henningsen, and H. Förstl, "Dysfunctional cognitions in idiopathic environmental intolerances (IEI)–an integrative psychiatric perspective," *Toxicology Letters*, vol. 171, no. 1-2, pp. 1–9, Jun. 2007.

[118] A. Aamland, K. Malterud, and E. L. Werner, "Patients with persistent medically unexplained physical symptoms: a descriptive study from Norwegian general practice," *BMC Family Practice*, vol. 15, no. 1, p. 107, Dec. 2014. [Online]. Available: https://bmcfampract.biomedcentral.com/articles/10.1186/1471-2296-15-107

[119] N. M. Eriksson and B. G. T. Stenberg, "Baseline prevalence of symptoms related to indoor environment," *Scandinavian Journal of Public Health*, vol. 34, no. 4, pp. 387–396, 2006.

[120] A. Schmoldt, H. F. Benthe, and G. Haberland, "Digitoxin metabolism by rat liver microsomes," *Biochemical Pharmacology*, vol. 24, no. 17, pp. 1639–1641, Sep. 1975.

[121] E. Palmquist, A.-S. Claeson, G. Neely, B. Stenberg, and S. Nordin, "Overlap in prevalence between various types of environmental intolerance," *International Journal of Hygiene and Environmental Health*, vol. 217, no. 4-5, pp. 427–434, May 2014.

[122] S. Hojo, S. Ishikawa, H. Kumano, M. Miyata, and K. Sakabe, "Clinical characteristics of physician-diagnosed patients with multiple chemical sensitivity in Japan," *International Journal of Hygiene and Environmental Health*, vol. 211, no. 5-6, pp. 682–689, Oct. 2008.

[123] T. To, S. Stanojevic, G. Moores, A. S. Gershon, E. D. Bateman, A. A. Cruz, and L.-P. Boulet, "Global asthma prevalence in adults: findings from the cross-sectional world health survey," *BMC public health*, vol. 12, p. 204, Mar. 2012.

[124] S. E. Wenzel, "Asthma phenotypes: the evolution from clinical to molecular approaches," *Nature Medicine*, vol. 18, no. 5, pp. 716–725, May 2012.

[125] H. Xia, Y. Li, and X. Lv, "MicroRNA-107 inhibits tumor growth and metastasis by targeting the BDNF-mediated PI3K/AKT pathway in human non-small lung cancer," *International Journal of Oncology*, vol. 49, no. 4, pp. 1325–1333, Oct. 2016.

[126] P. Ilmarinen, L. E. Tuomisto, and H. Kankaanranta, "Phenotypes, Risk Factors, and Mechanisms of Adult-Onset Asthma," *Mediators of Inflammation*, vol. 2015, p. 514868, 2015.

[127] E. Rönmark, A. Lindberg, L. Watson, and B. Lundbäck, "Outcome and severity of adult onset asthma–report from the obstructive lung disease in northern Sweden studies (OLIN)," *Respiratory Medicine*, vol. 101, no. 11, pp. 2370–2377, Nov. 2007.

[128] A. Sood, C. Qualls, M. Schuyler, A. Arynchyn, J. H. Alvarado, L. J. Smith, and D. R. Jacobs, "Adult-onset asthma becomes the dominant phenotype among women by age 40 years. the longitudinal CARDIA study," *Annals of the American Thoracic Society*, vol. 10, no. 3, pp. 188–197, Jun. 2013.

[129] L.-P. Boulet, "Influence of comorbid conditions on asthma," *The European Respiratory Journal*, vol. 33, no. 4, pp. 897–906, Apr. 2009.

[130] L.-P. Boulet and M.-v. Boulay, "Asthma-related comorbidities," *Expert Review of Respiratory Medicine*, vol. 5, no. 3, pp. 377–393, Jun. 2011.

[131] G. A. Stevens, G. M. Singh, Y. Lu, G. Danaei, J. K. Lin, M. M. Finucane, A. N. Bahalim, R. K. McIntire, H. R. Gutierrez, M. Cowan, C. J. Paciorek, F. Farzadfar, L. Riley, M. Ezzati, and Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index), "National, regional, and global trends in adult overweight and obesity prevalences," *Population Health Metrics*, vol. 10, no. 1, p. 22, Nov. 2012.

[132] K. Jameson, V. Amber, K. D'Oca, D. Mills, A. Giles, and B. Ambegaonkar, "Impact of lipid-lowering therapy on the prevalence of dyslipidaemia in patients at high-risk of cardiovascular events in UK primary care - a retrospective database study," *International Journal of Clinical Practice*, vol. 67, no. 12, pp. 1228–1237, Dec. 2013.

[133] A. S. Gershon, C. Wang, J. Guan, and T. To, "Burden of comorbidity in individuals with asthma," *Thorax*, vol. 65, no. 7, pp. 612–618, Jul. 2010.

[134] J. F. Maspero, C. H. Katelaris, W. W. Busse, M. Castro, J. Corren, B. E. Chipps, A. T. Peters, I. D. Pavord, L. B. Ford, L. Sher, K. F. Rabe, M. S. Rice, P. Rowe, Y. Lu, S. Harel, A. Jagerschmidt, A. H. Khan, S. Kamat,

G. Pirozzi, N. Amin, M. Ruddy, N. M. H. Graham, L. P. Mannent, and A. Teper, "Dupilumab Efficacy in Uncontrolled, Moderate-to-Severe Asthma with Self-Reported Chronic Rhinosinusitis," *The Journal of Allergy and Clinical Immunology. In Practice*, vol. 8, no. 2, pp. 527–539.e9, Feb. 2020.

[135] B. A. Senior, D. W. Kennedy, J. Tanabodee, H. Kroger, M. Hassab, and D. C. Lanza, "Long-term impact of functional endoscopic sinus surgery on asthma," *Otolaryngology–Head and Neck Surgery: Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*, vol. 121, no. 1, pp. 66–68, Jul. 1999.

[136] J. Manfreda, M. R. Becklake, M. R. Sears, M. Chan-Yeung, H. Dimich-Ward, H. C. Siersted, P. Ernst, L. Sweet, L. Van Til, D. M. Bowie, N. R. Anthonisen, and R. B. Tate, "Prevalence of asthma symptoms among adults aged 20-44 years in Canada," *CMAJ: Canadian Medical Association journal = journal de l'Association medicale canadienne*, vol. 164, no. 7, pp. 995–1001, Apr. 2001.

[137] M.-C. Tsai, H.-L. Lin, C.-C. Lin, H.-C. Lin, Y.-H. Chen, S. Pfeiffer, and H.-C. Lin, "Increased risk of concurrent asthma among patients with gastroesophageal reflux disease: a nationwide population-based study," *European Journal of Gastroenterology & Hepatology*, vol. 22, no. 10, pp. 1169–1173, Oct. 2010.

[138] N. Vakil, S. V. van Zanten, P. Kahrilas, J. Dent, R. Jones, and Global Consensus Group, "The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus," *The American Journal of Gastroenterology*, vol. 101, no. 8, pp. 1900–1920; quiz 1943, Aug. 2006.

[139] J. W. McCallister, J. P. Parsons, and J. G. Mastronarde, "The relationship between gastroesophageal reflux and asthma: an update," *Therapeutic Advances in Respiratory Disease*, vol. 5, no. 2, pp. 143–150, Apr. 2011.

[140] E. J. Williamson, A. J. Walker, K. Bhaskaran, S. Bacon, C. Bates, C. E. Morton, H. J. Curtis, A. Mehrkar, D. Evans, P. Inglesby, J. Cockburn, H. I. McDonald, B. MacKenna, L. Tomlinson, I. J. Douglas, C. T. Rentsch, R. Mathur, A. Y. S. Wong, R. Grieve, D. Harrison, H. Forbes, A. Schultze, R. Croker, J. Parry, F. Hester, S. Harper, R. Perera, S. J. W. Evans, L. Smeeth, and B. Goldacre, "Factors associated with COVID-19-related death using OpenSAFELY," *Nature*, vol. 584, no. 7821, pp. 430–436, Aug. 2020.

[141] J. L. Atkins, J. A. H. Masoli, J. Delgado, L. C. Pilling, C.-L. Kuo, G. A. Kuchel, and D. Melzer, "Preexisting Comorbidities Predicting COVID-19 and Mortality in the UK Biobank Community Cohort," *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, vol. 75, no. 11, pp. 2224–2230, Oct. 2020.

[142] N. G. Papadopoulos, I. Christodoulou, G. Rohde, I. Agache, C. Almqvist, A. Bruno, S. Bonini, L. Bont, A. Bossios, J. Bousquet, F. Braido, G. Brusselle, G. W. Canonica, K. H. Carlsen, P. Chanez, W. J. Fokkens, M. Garcia-Garcia, M. Gjomarkaj, T. Haahtela, S. T. Holgate, S. L. Johnston, G. Konstantinou, M. Kowalski, A. Lewandowska-Polak, K. Lødrup-Carlsen, M. Mäkelä, I. Malkusova, J. Mullol, A. Nieto, E. Eller, C. Ozdemir, P. Panzner, T. Popov, S. Psarras, E. Roumpedaki, M. Rukhadze, A. Stipic-Markovic, A. Todo Bom, E. Toskala, P. van Cauwenberge, C. van Drunen, J. B. Watelet, M. Xatzipsalti, P. Xepapadaki, and T. Zuberbier, "Viruses and bacteria in acute asthma exacerbations–a GA^2 LEN-DARE systematic review," *Allergy*, vol. 66, no. 4, pp. 458–468, Apr. 2011.

[143] I. Satia, R. Cusack, J. M. Greene, P. M. O'Byrne, K. J. Killian, and N. Johnston, "Prevalence and contribution of respiratory viruses in the community to rates of emergency department visits and hospitalizations with respiratory tract infections, chronic obstructive pulmonary disease and asthma," *PloS One*, vol. 15, no. 2, p. e0228544, 2020.

[144] X.-Y. Zheng, Y.-J. Xu, W.-J. Guan, and L.-F. Lin, "Regional, age and respiratory-secretion-specific prevalence of respiratory viruses associated with asthma exacerbation: a literature review," *Archives of Virology*, vol. 163, no. 4, pp. 845–853, Apr. 2018.

[145] D. P. Skoner, "Allergic rhinitis: Definition, epidemiology, pathophysiology, detection, and diagnosis," *Journal of Allergy and Clinical Immunology*, vol. 108, no. 1, pp. S2–S8, Jul. 2001. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0091674901944604

[146] Y.-G. Min, "The pathophysiology, diagnosis and treatment of allergic rhinitis," *Allergy, Asthma & Immunology Research*, vol. 2, no. 2, pp. 65–76, Apr. 2010.

[147] W. W. Storms, "Pharmacologic approaches to daytime and nighttime symptoms of allergic rhinitis," *The Journal of Allergy and Clinical Immunology*, vol. 114, no. 5 Suppl, pp. S146–153, Nov. 2004.

[148] R. Jankowski, D. T. Nguyen, M. Poussel, B. Chenuel, P. Gallet, and C. Rumeau, "Sinusology," *European Annals of Otorhinolaryngology, Head and Neck Diseases*, vol. 133, no. 4, pp. 263–268, Sep. 2016.

[149] W. David Boothe, J. A. Tarbox, and M. B. Tarbox, "Atopic Dermatitis: Pathophysiology," *Advances in Experimental Medicine and Biology*, vol. 1027, pp. 21–37, 2017.

[150] S. Takeuchi, H. Esaki, and M. Furue, "Epidemiology of atopic dermatitis in Japan," *The Journal of Dermatology*, vol. 41, no. 3, pp. 200–204, Mar. 2014.

[151] E. P. Rönmark, L. Ekerljung, J. Lötvall, G. Wennergren, E. Rönmark, K. Torén, and B. Lundbäck, "Eczema among adults: prevalence, risk factors and relation to airway diseases. Results from a large-scale population survey in Sweden," *The British Journal of Dermatology*, vol. 166, no. 6, pp. 1301–1308, Jun. 2012.

[152] C. A. Akdis, M. Akdis, T. Bieber, C. Bindslev-Jensen, M. Boguniewicz, P. Eigenmann, Q. Hamid, A. Kapp, D. Y. M. Leung, J. Lipozencic, T. A. Luger, A. Muraro, N. Novak, T. a. E. Platts-Mills, L. Rosenwasser, A. Scheynius, F. E. R. Simons, J. Spergel, K. Turjanmaa, U. Wahn, S. Weidinger, T. Werfel, T. Zuberbier, European Academy of Allergology, and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Group, "Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report," *Allergy*, vol. 61, no. 8, pp. 969–987, Aug. 2006.

[153] L. Bjermer, "Time for a paradigm shift in asthma treatment: from relieving bronchospasm to controlling systemic inflammation," *The Journal of Allergy and Clinical Immunology*, vol. 120, no. 6, pp. 1269–1275, Dec. 2007.

[154] "Acupuncture treatment of a patient with persistent allergic rhinitis complicated by rhinosinusitis and asthma," vol. 2011, p. 798081, 2011.

[155] Y.-Y. Hsieh, C.-C. Chang, C.-M. Hsu, L. Wan, S.-Y. Chen, W.-H. Lin, and F.-J. Tsai, "JAK-1 rs2780895 C-related genotype and allele but not JAK-1 rs10789166, rs4916008, rs2780885, rs17127114, and rs3806277 are associated with higher susceptibility to asthma," *Genetic Testing and Molecular Biomarkers*, vol. 15, no. 12, pp. 841–847, Dec. 2011.

[156] S. M. McCormick and N. M. Heller, "Commentary: IL-4 and IL-13 receptors and signaling," *Cytokine*, vol. 75, no. 1, pp. 38–50, Sep. 2015.

[157] F.-Q. Wen, T. Kohyama, X. Liu, Y. K. Zhu, H. Wang, H. J. Kim, T. Kobayashi, S. Abe, J. R. Spurzem, and S. I. Rennard, "Interleukin-4- and interleukin-13-enhanced transforming growth factor-beta2 production in cultured human bronchial epithelial cells is attenuated by interferon-gamma," *American Journal of Respiratory Cell and Molecular Biology*, vol. 26, no. 4, pp. 484–490, Apr. 2002.

[158] S.-C. Lin, J.-J. Huang, J.-Y. Wang, H.-C. Chuang, B.-L. Chiang, and Y.-L. Ye, "Upregulated thymic stromal lymphopoietin receptor expression in children with asthma," *European Journal of Clinical Investigation*, vol. 46, no. 6, pp. 511–519, Jun. 2016.

[159] D. M. André, M. C. Calixto, C. Sollon, E. C. Alexandre, L. O. Leiria, N. Tobar, G. F. Anhê, and E. Antunes, "Therapy with resveratrol attenuates obesity-associated allergic airway inflammation in mice," *International Immunopharmacology*, vol. 38, pp. 298–305, Sep. 2016.

[160] A. S. Williams, D. I. Kasahara, N. G. Verbout, A. V. Fedulov, M. Zhu, H. Si, A. P. Wurmbrand, C. Hug, B. Ranscht, and S. A. Shore, "Role of the adiponectin binding protein, T-cadherin (Cdh13), in allergic airways responses in mice," *PloS One*, vol. 7, no. 7, p. e41088, 2012.

[161] D. G. Hardie, F. A. Ross, and S. A. Hawley, "AMPK: a nutrient and energy sensor that maintains energy homeostasis," *Nature Reviews. Molecular Cell Biology*, vol. 13, no. 4, pp. 251–262, Mar. 2012.

[162] B. Tang, P. Kang, J. Guo, L. Zhu, Q. Xu, Q. Gao, H. Zhang, and H. Wang, "[Effects of mitochondrial aldehyde dehydrogenase 2 on autophagy-associated proteins in neonatal rat myocardial fibroblasts cultured in high glucose]," *Nan Fang Yi Ke Da Xue Xue Bao = Journal of Southern Medical University*, vol. 39, no. 5, pp. 523–527, May 2019.

[163] T. D. Gilmore, "Introduction to NF-kappaB: players, pathways, perspectives," *Oncogene*, vol. 25, no. 51, pp. 6680–6684, Oct. 2006.

[164] J. H. Bae, H. J. Choi, K. I. K. Cho, L. K. Kim, J. S. Kwon, and Y. M. Cho, "Glucagon-Like Peptide-1 Receptor Agonist Differentially Affects Brain Activation in Response to Visual Food Cues in Lean and Obese Individuals with Type 2 Diabetes Mellitus," *Diabetes & Metabolism Journal*, vol. 44, no. 2, pp. 248–259, Apr. 2020.

[165] C. Domingo, O. Palomares, D. A. Sandham, V. J. Erpenbeck, and P. Altman, "The prostaglandin D2 receptor 2 pathway in asthma: a key player in airway inflammation," *Respiratory Research*, vol. 19, no. 1, p. 189, Sep. 2018.

[166] S. G. Ward, "New drug targets in inflammation: efforts to expand the anti-inflammatory armoury," *British Journal of Pharmacology*, vol. 153 Suppl 1, pp. S5–6, Mar. 2008.

[167] D. A. Sykes, M. E. Bradley, D. M. Riddy, E. Willard, J. Reilly, A. Miah, C. Bauer, S. J. Watson, D. A. Sandham, G. Dubois, and S. J. Charlton, "Fevipiprant (QAW039), a Slowly Dissociating CRTh2 Antagonist with the Potential for Improved Clinical Efficacy," *Molecular Pharmacology*, vol. 89, no. 5, pp. 593–605, May 2016.

[168] G. G. Chen, T. W. Lee, H. Xu, J. H. Y. Yip, M. Li, T. S. K. Mok, and A. P. C. Yim, "Increased inducible nitric oxide synthase in lung carcinoma of smokers," *Cancer*, vol. 112, no. 2, pp. 372–381, Jan. 2008.

[169] N.-R. Shin, C. Kim, C.-S. Seo, J.-W. Ko, Y.-K. Cho, I.-S. Shin, and J.-S. Kim, "Galgeun-tang Attenuates Cigarette Smoke and Lipopolysaccharide Induced Pulmonary Inflammation via I$\kappa\beta\alpha$/NF-κB Signaling," *Molecules (Basel, Switzerland)*, vol. 23, no. 10, Sep. 2018.

[170] N. J. Nilsen, G. I. Vladimer, J. Stenvik, M. P. A. Orning, M. V. Zeid-Kilani, M. Bugge, B. Bergstroem, J. Conlon, H. Husebye, A. G. Hise, K. A. Fitzgerald, T. Espevik, and E. Lien, "A role for the adaptor proteins TRAM and TRIF in toll-like receptor 2 signaling," *The Journal of Biological Chemistry*, vol. 290, no. 6, pp. 3209–3222, Feb. 2015.

[171] J. Zhu, S. D. Message, P. Mallia, T. Kebadze, M. Contoli, C. K. Ward, E. S. Barnathan, M. A. Mascelli, O. M. Kon, A. Papi, L. A. Stanciu, M. R. Edwards, P. K. Jeffery, and S. L. Johnston, "Bronchial mucosal IFN-α/β and pattern recognition receptor expression in patients with experimental rhinovirus-induced asthma exacerbations," *The Journal of Allergy and Clinical Immunology*, vol. 143, no. 1, pp. 114–125.e4, Jan. 2019.

[172] Y. Huo, L. Guan, J. Xu, L. Zhou, and R. Chen, "Tiotropium inhibits methacholine-induced extracellular matrix production via β-catenin signaling in human airway smooth muscle cells," *International Journal of Chronic Obstructive Pulmonary Disease*, vol. 13, pp. 1469–1481, 2018.

[173] A. Vatrella, I. Fabozzi, C. Calabrese, R. Maselli, and G. Pelaia, "Dupilumab: a novel treatment for asthma," *Journal of Asthma and Allergy*, vol. 7, pp. 123–130, 2014.

[174] I. I. M. Tromp, O. H. Franco, E. H. van den Hooven, A. C. Heijboer, V. W. V. Jaddoe, L. Duijts, J. C. de Jongste, H. A. Moll, and J. C. Kiefte-de Jong, "25-Hydroxyvitamin D concentrations, asthma and eczema in childhood: The generation R study," *Clinical Nutrition (Edinburgh, Scotland)*, vol. 37, no. 1, pp. 169–176, Feb. 2018.

[175] O. Aguilera, C. Peña, J. M. García, M. J. Larriba, P. Ordóñez-Morán, D. Navarro, A. Barbáchano, I. López de Silanes, E. Ballestar, M. F. Fraga, M. Esteller, C. Gamallo, F. Bonilla, J. M. González-Sancho, and A. Muñoz, "The Wnt antagonist DICKKOPF-1 gene is induced by 1alpha,25-dihydroxyvitamin D3 associated to the differentiation of human colon cancer cells," *Carcinogenesis*, vol. 28, no. 9, pp. 1877–1884, Sep. 2007.

[176] Y. Huang, L. Wang, X.-X. Jia, X.-X. Lin, and W.-X. Zhang, "Vitamin D alleviates airway remodeling in asthma by down-regulating the activity of Wnt/β-catenin signaling pathway," *International Immunopharmacology*, vol. 68, pp. 88–94, Mar. 2019.

[177] H. H. Pua and K. M. Ansel, "MicroRNA regulation of allergic inflammation and asthma," *Current Opinion in Immunology*, vol. 36, pp. 101–108, Oct. 2015.

[178] L. Huang, Z. Huang, Y. Fan, L. He, M. Ye, K. Shi, B. Ji, J. Huang, Y. Wang, and Q. Li, "FOXC1 promotes proliferation and epithelial-mesenchymal transition in cervical carcinoma through the PI3K-AKT signal pathway," *American Journal of Translational Research*, vol. 9, no. 3, pp. 1297–1306, 2017.

[179] I. Kuwabara, Y. Kuwabara, R.-Y. Yang, M. Schuler, D. R. Green, B. L. Zuraw, D. K. Hsu, and F.-T. Liu, "Galectin-7 (PIG1) exhibits pro-apoptotic function through JNK activation and mitochondrial cytochrome c release," *The Journal of Biological Chemistry*, vol. 277, no. 5, pp. 3487–3497, Feb. 2002.

[180] C. Vizetto-Duarte, L. Custódio, K. N. Gangadhar, J. H. G. Lago, C. Dias, A. M. Matos, N. Neng, J. M. F. Nogueira, L. Barreira, F. Albericio, A. P. Rauter, and J. Varela, "Isololiolide, a carotenoid metabolite isolated from the brown alga Cystoseira tamariscifolia, is cytotoxic and able to induce apoptosis in hepatocarcinoma cells through caspase-3 activation, decreased Bcl-2 levels, increased p53 expression and PARP cleavage," *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, vol. 23, no. 5, pp. 550–557, May 2016.

[181] C. Villeneuve, L. Baricault, L. Canelle, N. Barboule, C. Racca, B. Monsarrat, T. Magnaldo, and F. Larminat, "Mitochondrial proteomic approach reveals galectin-7 as a novel BCL-2 binding protein in human cells," *Molecular Biology of the Cell*, vol. 22, no. 7, pp. 999–1013, Apr. 2011.

[182] X. Sun and W. Zhang, "Silencing of Gal-7 inhibits TGF-β1-induced apoptosis of human airway epithelial cells through JNK signaling pathway," *Experimental Cell Research*, vol. 375, no. 2, pp. 100–105, Feb. 2019.

[183] O. De Wever, W. Westbroek, A. Verloes, N. Bloemen, M. Bracke, C. Gespach, E. Bruyneel, and M. Mareel, "Critical role of N-cadherin in myofibroblast invasion and migration in vitro stimulated by colon-cancer-cell-derived TGF-beta or wounding," *Journal of Cell Science*, vol. 117, no. Pt 20, pp. 4691–4703, Sep. 2004.

[184] P. Lachapelle, M. Li, J. Douglass, and A. Stewart, "Safer approaches to therapeutic modulation of TGF-β signaling for respiratory disease," *Pharmacology & Therapeutics*, vol. 187, pp. 98–113, Jul. 2018.

[185] F. Ding, Z. Fu, and B. Liu, "Lipopolysaccharide Exposure Alleviates Asthma in Mice by Regulating Th1/Th2 and Treg/Th17 Balance," *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, vol. 24, pp. 3220–3229, May 2018.

[186] Y. Zhu, D. Mao, W. Gao, G. Han, and H. Hu, "Analysis of lncRNA Expression in Patients With Eosinophilic and Neutrophilic Asthma Focusing on LNC_000127," *Frontiers in Genetics*, vol. 10, p. 141, 2019.

[187] Y.-Y. Qiu, Y. Wu, M.-J. Lin, T. Bian, Y.-L. Xiao, and C. Qin, "LncRNA-MEG3 functions as a competing endogenous RNA to regulate Treg/Th17 balance in patients with asthma by targeting microRNA-17/ RORγt," *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, vol. 111, pp. 386–394, Mar. 2019.

[188] F. Yuan, R. Liu, M. Hu, X. Rong, L. Bai, L. Xu, Y. Mao, H. Hasimu, Y. Sun, and J. He, "JAX2, an ethanol extract of Hyssopus cuspidatus Boriss, can prevent bronchial asthma by inhibiting MAPK/NF-κB inflammatory signaling," *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, vol. 57, pp. 305–314, Apr. 2019.

[189] S. E. Dahlén, "Pharmacological characterization of leukotriene receptors," *American Journal of Respiratory and Critical Care Medicine*, vol. 161, no. 2 Pt 2, pp. S41–45, Feb. 2000.

[190] H. K. T. Trinh, D.-H. Suh, T. V. T. Nguyen, Y. Choi, H.-S. Park, and Y. S. Shin, "Characterization of cysteinyl leukotriene-related receptors and their interactions in a mouse model of asthma," *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, vol. 141, pp. 17–23, Feb. 2019.

[191] S. Grundy, J. Plumb, M. Kaur, D. Ray, and D. Singh, "Additive anti-inflammatory effects of corticosteroids and phosphodiesterase-4 inhibitors in COPD CD8 cells," *Respiratory Research*, vol. 17, p. 9, Jan. 2016.

[192] N. Krug, T. Tschernig, K. Balke, V. J. Erpenbeck, L. Meyer, U. Bode, J. M. Hohlfeld, R. Pabst, and H. Fabel, "Enhanced expression of fas ligand

(CD95L) on T cells after segmental allergen provocation in asthma," *The Journal of Allergy and Clinical Immunology*, vol. 103, no. 4, pp. 649–655, Apr. 1999.

[193] P. H. Krammer, R. Arnold, and I. N. Lavrik, "Life and death in peripheral T cells," *Nature Reviews. Immunology*, vol. 7, no. 7, pp. 532–542, Jul. 2007.

[194] Y. Liu, B. Ji, G. Zhao, H. Su, Y. Ge, J. Dai, Y. Lu, R. Sakurai, and V. K. Rehan, "Protective effect of electro-acupuncture at maternal different points on perinatal nicotine exposure-induced pulmonary dysplasia in offspring based on HPA axis and signal transduction pathway," *Biochemical and Biophysical Research Communications*, vol. 505, no. 2, pp. 586–592, Oct. 2018.

[195] V. K. Rehan and J. S. Torday, "PPARγ Signaling Mediates the Evolution, Development, Homeostasis, and Repair of the Lung," *PPAR research*, vol. 2012, p. 289867, 2012.

[196] S. Miyamoto, N. Hattori, T. Senoo, Y. Onari, H. Iwamoto, M. Kanehara, N. Ishikawa, K. Fujitaka, Y. Haruta, H. Murai, A. Yokoyama, and N. Kohno, "Intra-airway administration of small interfering RNA targeting plasminogen activator inhibitor-1 attenuates allergic asthma in mice," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 301, no. 6, pp. L908–916, Dec. 2011.

[197] K. Mukai, K. Obata, Y. Tsujimura, and H. Karasuyama, "New insights into the roles for basophils in acute and chronic allergy," *Allergology International: Official Journal of the Japanese Society of Allergology*, vol. 58, no. 1, pp. 11–19, Mar. 2009.

[198] Y. Liu, M. Zhu, K. Nishida, T. Hirano, and W. Zhang, "An essential role for RasGRP1 in mast cell function and IgE-mediated allergic response," *The Journal of Experimental Medicine*, vol. 204, no. 1, pp. 93–103, Jan. 2007.

[199] G. Liu and H. Yang, "Modulation of macrophage activation and programming in immunity," *Journal of Cellular Physiology*, vol. 228, no. 3, pp. 502–512, Mar. 2013.

[200] C. A. Sabatos, S. Chakravarti, E. Cha, A. Schubart, A. Sánchez-Fueyo, X. X. Zheng, A. J. Coyle, T. B. Strom, G. J. Freeman, and V. K. Kuchroo, "Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance," *Nature Immunology*, vol. 4, no. 11, pp. 1102–1110, Nov. 2003.

[201] J.-L. Casanova, L. Abel, and L. Quintana-Murci, "Human TLRs and IL-1Rs in host defense: natural insights from evolutionary, epidemiological, and clinical genetics," *Annual Review of Immunology*, vol. 29, pp. 447–491, 2011.

[202] S. K. Shenoy and R. J. Lefkowitz, "Seven-transmembrane receptor signaling through beta-arrestin," *Science's STKE: signal transduction knowledge environment*, vol. 2005, no. 308, p. cm10, Nov. 2005.

[203] S. S. Athari, "Targeting cell signaling in allergic asthma," *Signal Transduction and Targeted Therapy*, vol. 4, p. 45, 2019.

[204] H. Wang, K. Liu, M. Geng, P. Gao, X. Wu, Y. Hai, Y. Li, Y. Li, L. Luo, J. D. Hayes, X. J. Wang, and X. Tang, "RXRα inhibits the NRF2-ARE signaling pathway through a direct interaction with the Neh7 domain of NRF2," *Cancer Research*, vol. 73, no. 10, pp. 3097–3108, May 2013.

[205] C. J. Koziol-White and R. A. Panettieri, "Modulation of Bronchomotor Tone Pathways in Airway Smooth Muscle Function and Bronchomotor Tone in Asthma," *Clinics in Chest Medicine*, vol. 40, no. 1, pp. 51–57, Mar. 2019.

[206] "Computational analysis of multimorbidity between asthma, eczema and rhinitis," *PloS One*, vol. 12, no. 6, p. e0179125.

[207] A. Dehghan, "Genome-Wide Association Studies," *Methods in Molecular Biology (Clifton, N.J.)*, vol. 1793, pp. 37–49, 2018.

[208] R. J. Klein, X. Xu, S. Mukherjee, J. Willis, and J. Hayes, "Successes of genome-wide association studies," *Cell*, vol. 142, no. 3, pp. 350–351; author reply 353–355, Aug. 2010.

[209] Y. Liu, Y. Liang, and D. Wishart, "PolySearch2: a significantly improved text-mining system for discovering associations between human diseases, genes, drugs, metabolites, toxins and more," *Nucleic Acids Research*, vol. 43, no. W1, pp. W535–542, Jul. 2015.

[210] A. D. Yates, P. Achuthan, W. Akanni, J. Allen, J. Allen, J. Alvarez-Jarreta, M. R. Amode, I. M. Armean, A. G. Azov, R. Bennett, J. Bhai, K. Billis, S. Boddu, J. C. Marugán, C. Cummins, C. Davidson, K. Dodiya, R. Fatima, A. Gall, C. G. Giron, L. Gil, T. Grego, L. Haggerty, E. Haskell, T. Hourlier, O. G. Izuogu, S. H. Janacek, T. Juettemann, M. Kay, I. Lavidas, T. Le, D. Lemos, J. G. Martinez, T. Maurel, M. McDowall, A. McMahon, S. Mohanan, B. Moore, M. Nuhn, D. N. Oheh, A. Parker, A. Parton, M. Patricio, M. P. Sakthivel, A. I. Abdul Salam, B. M. Schmitt, H. Schuilenburg, D. Sheppard, M. Sycheva, M. Szuba, K. Taylor, A. Thormann, G. Threadgold, A. Vullo, B. Walts, A. Winterbottom, A. Zadissa, M. Chakiachvili, B. Flint, A. Frankish, S. E. Hunt, G. IIsley, M. Kostadima, N. Langridge, J. E. Loveland, F. J. Martin, J. Morales, J. M. Mudge, M. Muffato, E. Perry, M. Ruffier, S. J. Trevanion, F. Cunningham, K. L. Howe, D. R. Zerbino, and P. Flicek, "Ensembl 2020," *Nucleic Acids Research*, vol. 48, no. D1, pp. D682–D688, Jan. 2020.

[211] UniProt Consortium, "Reorganizing the protein space at the Universal Protein Resource (UniProt)," *Nucleic Acids Research*, vol. 40, no. Database issue, pp. D71–75, Jan. 2012.

[212] ——, "The Universal Protein Resource (UniProt) 2009," *Nucleic Acids Research*, vol. 37, no. Database issue, Jan. 2009.

[213] B. E. Suzek, H. Huang, P. McGarvey, R. Mazumder, and C. H. Wu, "UniRef: comprehensive and non-redundant UniProt reference clusters," *Bioinformatics (Oxford, England)*, vol. 23, no. 10, pp. 1282–1288, May 2007.

[214] K. D. Pruitt, T. Tatusova, and D. R. Maglott, "NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins," *Nucleic Acids Research*, vol. 35, no. Database issue, pp. D61–65, Jan. 2007.

[215] B. Jassal, L. Matthews, G. Viteri, C. Gong, P. Lorente, A. Fabregat, K. Sidiropoulos, J. Cook, M. Gillespie, R. Haw, F. Loney, B. May, M. Milacic, K. Rothfels, C. Sevilla, V. Shamovsky, S. Shorser, T. Varusai, J. Weiser, G. Wu, L. Stein, H. Hermjakob, and P. D'Eustachio, "The reactome pathway knowledgebase," *Nucleic Acids Research*, vol. 48, no. D1, Jan. 2020.

[216] D. Croft, G. O'Kelly, G. Wu, R. Haw, M. Gillespie, L. Matthews, M. Caudy, P. Garapati, G. Gopinath, B. Jassal, S. Jupe, I. Kalatskaya, S. Mahajan, B. May, N. Ndegwa, E. Schmidt, V. Shamovsky, C. Yung, E. Birney, H. Hermjakob, P. D'Eustachio, and L. Stein, "Reactome: a database of reactions, pathways and biological processes," *Nucleic Acids Research*, vol. 39, no. Database issue, pp. D691–697, Jan. 2011.

[217] M. Kanehisa, M. Furumichi, M. Tanabe, Y. Sato, and K. Morishima, "KEGG: new perspectives on genomes, pathways, diseases and drugs," *Nucleic Acids Research*, vol. 45, no. D1, pp. D353–D361, Jan. 2017.

[218] M. H. Schaefer, J.-F. Fontaine, A. Vinayagam, P. Porras, E. E. Wanker, and M. A. Andrade-Navarro, "HIPPIE: Integrating protein interaction networks with experiment based quality scores," *PloS One*, vol. 7, no. 2, p. e31826, 2012.

[219] K. Breuer, A. K. Foroushani, M. R. Laird, C. Chen, A. Sribnaia, R. Lo, G. L. Winsor, R. E. W. Hancock, F. S. L. Brinkman, and D. J. Lynn, "InnateDB: systems biology of innate immunity and beyond–recent updates and continuing curation," *Nucleic Acids Research*, vol. 41, no. Database issue, pp. D1228–1233, Jan. 2013.

[220] D. J. Lynn, G. L. Winsor, C. Chan, N. Richard, M. R. Laird, A. Barsky, J. L. Gardy, F. M. Roche, T. H. W. Chan, N. Shah, R. Lo, M. Naseer, J. Que, M. Yau, M. Acab, D. Tulpan, M. D. Whiteside, A. Chikatamarla, B. Mah, T. Munzner, K. Hokamp, R. E. W. Hancock, and F. S. L. Brinkman, "InnateDB: facilitating systems-level analyses of the mammalian innate immune response," *Molecular Systems Biology*, vol. 4, p. 218, 2008.

[221] F. Nippert, R. Schreckenberg, and K.-D. Schlüter, "Isolation and Cultivation of Adult Rat Cardiomyocytes," *Journal of Visualized Experiments*, no. 128, p. 56634, Oct. 2017. [Online]. Available: https://www.jove.com/video/56634/isolation-and-cultivation-of-adult-rat-cardiomyocytes

[222] E. Glaab, A. Baudot, N. Krasnogor, R. Schneider, and A. Valencia, "Enrichnet: network-based gene set enrichment analysis," *Bioinformatics*, vol. 28, no. 18, pp. i451–i457, 2012.

[223] A. N. Tegge, N. Sharp, and T. Murali, "Xtalk: a path-based approach for identifying crosstalk between signaling pathways," *Bioinformatics*, vol. 32, no. 2, pp. 242–251, 2016.

[224] S. C. Payne, L. Borish, and J. W. Steinke, "Genetics and phenotyping in chronic sinusitis," *Journal of Allergy and Clinical Immunology*, vol. 128, no. 4, pp. 710–720, Oct. 2011. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0091674911008463

[225] M. Frieri, "Asthma linked with rhinosinusitis: An extensive review," *Allergy & rhinology*, vol. 5, no. 1, pp. ar–2014, 2014.

[226] A. Laulajainen-Hongisto, A. Lyly, T. Hanif, K. Dhaygude, M. Kankainen, R. Renkonen, K. Donner, P. Mattila, T. Jartti, J. Bousquet *et al.*, "Genomics of asthma, allergy and chronic rhinosinusitis: novel concepts and relevance in airway mucosa," *Clinical and translational allergy*, vol. 10, no. 1, pp. 1–17, 2020.

[227] A. Argyrou, E. Legaki, C. Koutserimpas, M. Gazouli, I. Papaconstantinou, G. Gkiokas, and G. Karamanolis, "Risk factors for gastroesophageal reflux

disease and analysis of genetic contributors," *World journal of clinical cases*, vol. 6, no. 8, p. 176, 2018.

[228] S.-P. Hu, G.-B. Zhou, J.-A. Luan, Y.-P. Chen, D.-W. Xiao, Y.-J. Deng, L.-Q. Huang, and K.-L. Cai, "Polymorphisms of hla-a and hla-b genes in genetic susceptibility to esophageal carcinoma in chaoshan han chinese," *Diseases of the Esophagus*, vol. 23, no. 1, pp. 46–52, 2010.

[229] J. Sidney, B. Peters, N. Frahm, C. Brander, and A. Sette, "Hla class i supertypes: a revised and updated classification," *BMC immunology*, vol. 9, no. 1, pp. 1–15, 2008.

[230] M. M. Kchir, W. Hamdi, L. Laadhar, S. Kochbati, D. Kaffel, K. Saadellaoui, H. Lahmar, M. M. Ghannouchi, D. Azzouz, L. Daoud *et al.*, "Hla-b, dr and dq antigens polymorphism in tunisian patients with ankylosing spondylitis (a case–control study)," *Rheumatology international*, vol. 30, no. 7, pp. 933–939, 2010.

[231] M. P. Nikolova and M. S. Chavali, "Metal oxide nanoparticles as biomedical materials," *Biomimetics*, vol. 5, no. 2, p. 27, 2020.

[232] A. Kelly and J. Trowsdale, "Genetics of antigen processing and presentation," *Immunogenetics*, vol. 71, no. 3, pp. 161–170, 2019.

[233] E. S. Dellon, "Eosinophilic Esophagitis," *Gastroenterology Clinics of North America*, vol. 42, no. 1, pp. 133–153, Mar. 2013. [Online]. Available: https://linkinghub.elsevier.com/retrieve/pii/S0889855312001306

[234] J. Günther and H.-M. Seyfert, "The first line of defence: insights into mechanisms and relevance of phagocytosis in epithelial cells," in *Seminars in immunopathology*, vol. 40, no. 6. Springer, 2018, pp. 555–565.

[235] H. Wang, L. Pan, and Z. Liu, "Neutrophils as a protagonist and target in chronic rhinosinusitis," *Clinical and experimental otorhinolaryngology*, vol. 12, no. 4, p. 337, 2019.

[236] G. Boeckxstaens, "the pathophysiology of gastro-oesophageal reflux disease," *Alimentary pharmacology & therapeutics*, vol. 26, no. 2, pp. 149–160, 2007.

[237] J. H. Kim and Y. J. Jang, "Role of natural killer cells in airway inflammation," *Allergy, asthma & immunology research*, vol. 10, no. 5, p. 448, 2018.

[238] Q. Hamid and M. Tulic, "Immunobiology of asthma," *Annual review of physiology*, vol. 71, pp. 489–507, 2009.

[239] K. D. Bardhan, V. Strugala, and P. W. Dettmar, "Reflux revisited: advancing the role of pepsin," *International journal of otolaryngology*, vol. 2012, 2012.

[240] S. Kollnberger, A. Chan, M.-Y. Sun, L. Ye Chen, C. Wright, K. di Gleria, A. McMichael, and P. Bowness, "Interaction of hla-b27 homodimers with kir3dl1 and kir3dl2, unlike hla-b27 heterotrimers, is independent of the sequence of bound peptide," *European journal of immunology*, vol. 37, no. 5, pp. 1313–1322, 2007.

[241] N. S. Chaimowitz and L. R. Forbes, "Human diseases caused by impaired signal transducer and activator of transcription and janus kinase signaling," *Current opinion in pediatrics*, vol. 31, no. 6, pp. 843–850, 2019.

[242] V. Tysoe-Calnon, J. Grundy, and S. Perkins, "Molecular comparisons of the β 2-microglobulin-binding site in class i major-histocompatibility-complex α-chains and proteins of related sequences," *Biochemical Journal*, vol. 277, no. 2, pp. 359–369, 1991.

[243] J. Geng, J. D. Altman, S. Krishnakumar, and M. Raghavan, "Empty conformers of hla-b preferentially bind cd8 and regulate cd8+ t cell function," *Elife*, vol. 7, p. e36341, 2018.

[244] C. Ogris, D. Guala, M. Kaduk, and E. L. Sonnhammer, "Funcoup 4: new species, data, and visualization," *Nucleic acids research*, vol. 46, no. D1, pp. D601–D607, 2018.

[245] B. T. Sherman, R. A. Lempicki *et al.*, "Systematic and integrative analysis of large gene lists using david bioinformatics resources," *Nature protocols*, vol. 4, no. 1, p. 44, 2009.

An Appendix

Table 1: Gene list retrieved from poly search database

Asthma	**Sinusitis**	**Rhinosinusitis**	**Gastroesophageal reflux disease**
A disintegrin and metalloprotease 33	16.1	C2orf86	EPH receptor B4
DVS27	"COX VIII-L"	OSF 2	CA IX
ALRH	Restin	CST-1	C-erb B2
DER-4		LUNX	ANX-1
GSDML		B cell differentiation factor gamma	CDC21
ORM1 (S. cerevisiae)-like 3		ALRH	CDABP0042
Lymphotoxin-beta		Activation inducer molecule	B lymphoma Mo-MLV insertion region (mouse)
39 kDa synovial protein		ABC35	COX-2
B cell differentiation factor gamma		TSLP	KLK-1
NR1F1		DVS27	ATP-dependent helicase SMARCA4

DENN/MADD domain containing 1B	"COX VIII-L"	Leukolysin
CTLA 8	Pulmonary function	Breast normal epithelial cell associated serine protease
5 lipoxygenase	COX-2	Hydroxyaryl protein kinase
CXCL12	CD154	FKHL-5
FKSG-9	PTC bitter taste receptor	CD151
ASRT-2	CD284	
B cell differentiation factor 1	B cell differentiation factor 1	
T box 21		
CYSLT-1		
Pulmonary function		
Catabolin		
TSLP		
Leukolysin		
Blastokinin		
ASRT-1		
OSF 2		
582J2.1		
AG-2 protein		
VEGF		
KIF-3		
ADRB-2		
CAMP specific phosphodiesterase variant PDE4A-10		

ZPBP-2

MIRN410

DEGS

LTC4 synthase

AZ3B

MUC-5

ABP-280

IRF-2

BM-040

CD14

CL100

CCL11

ADCY-9

CYSLT-2

ATOD-4

GCCR

B TCGF

ATP-dependent heli-

case SMARCA4

MUC19

FOXA-2

COX-2

CD126

IPO-B

DPL-2

BLAST 2

CC chemokine IMAC

CD87

3-10C

26 kd protein

Antigen CD48

FAM13A1	
A disintegrin and metalloproteinase domain 8	
GIMAP-4	
FTSJD2	
DPD-1	
DPDE-4	
STAT-4	
GIMAP-5	
FAM117C	
CD66c	
CARD-7	
IL-10-related T-cell-derived-inducible factor	
CD169	
Prostanoid TP receptor	
D12S1644	
CD284	
Oncogene PIM2	
EPHX	
COX-1	
HMOX-1	
GON-10	
Ets transcription factor PDEF	
C-C CKR-3	
Breast carcinoma associated antigen DF3	

AF-1	
Glia-derived nexin	
CSF-3	
DMTase	
CRF-1	
3'5' cyclic nucleotide phosphodiesterase 4D splice variant 1	
NRP-2	
MFAP-4	
MED22	
G protein coupled receptor 65	
CCAAT-box binding transcription factor	
39S ribosomal protein L44 mitochondrial	
26S proteasome non ATPase regulatory subunit 3	
ORAOV-2	
LUNX	
LAMA-1	
ELP-2	
ARG-2	
AML-3	
10 kDa interferon-gamma-induced protein	
CLCA-1	

ADP ribosyl cyclase

Table 2: Genes list retrieved from uniport

Asthma	**Rhinosinusitis**	**Sinusitis**	**Gastroesophageal reflux**
ORMDL3	IL22RA1	DNAI1	MECP2
ADAM33		CCDC151	MSR1
PLA2G7		CCDC39	TP53
DPP10		ZMYND10	GPX7
DENND1B		DNAH1	ALDH18A1
TBX21		IL12RB2	SALL1
MUC7		IL4R	GEMIN4
NPSR1		HYDIN	ASCC1
CDHR3		DNAH5	STAG1
IL4R		DNAAF1	CTHRC1
IRAK3		RSPH4A	LYRM4
MYLK		CCDC114	NEXMIF
CHI3L1		CCDC40	TSPYL1
MS4A2		DNAH11	
IL13		DNAL1	
RASGRP4		DNAH9	
IFNGR1		TTC25	
PTGDR		LRRC6	
PHF11		DNAI2	
CHIA		DNAAF6	
ADRB2		DNAAF5	
HNMT		DNAAF4	
CYSLTR1		CCDC103	
SMRP1		ARMC4	
S100A12		SPAG1	
CLCA1		RSPH9	
BCL11B		CCDC65	

IL17F	DNAAF2
IL12RB2	CFAP298
PRKCZ	DNAAF3
CLC	CFAP300
ADCY9	LRRC56
HAVCR1	
IL27	
NLRP3	
PIK3CG	
ARG2	
IL33	
ADAM33	
CCL4	
AAA1	
GSTP1	

Table 3: Gene list retrieved from ensemble

Rhinosinusitis	**Sinusitis**	**Gastroesophageal reflux disease**	**Asthma**
HLA-DQA1	DNAH8-AS1, DNAH8	AFF3	TLR1
IL33	DNAH11	NIPBL	SPATS2L
HLA-DQA1	TNNI3, DNAAF3	APOB	PRKCQ, PFKFB3, SFMBT2
ALOX15	RSPH4A	NIPBL	AKAP6
CFTR	CCDC40	SMD1	HLA-DRB1
	DNAAF1	MECP2	HLA-B
	BLNK	OLIG2, C21orf62	ITSN2, C2orf84, NCOA1

DNAI1	MICA, HLA-B	IL13, RAD50, IL4
RSPH1	CCKBR, CNGA4	HLA-DRB1, HLA-DQA1
DNAL1	MAD1L1, AK127048, ELFN1	
DNAAF5, PRKAR1B	RBM6, MON1A	
DNAH8	SLCO3A1, CRTC3, RP11-387D10.2	
RPGR	AK098570, LSP1P3	
CCDC40	DPYD	
CDCA7L, DNAH11	PDE4B, U4	
DNAAF2	KLHL26, CRTC1, TMEM59L	
LRRC6	NRIP1	
DNAH11	MICA, HLA-B	
DNAH5	GRM8	
CCDC39	LOC285819, BTN1A1, BTN2A1	
DNAH11	ANO10, SNRK	
CCDC40	PGPEP1, LSM4	
DNAAF1	MICA, HLA-B	
DRC1	MICA, HLA-B	
DNAL1	ADAMTS17, RNA5SP402, AC022819.3, CTD-3076O17.1	
DNAAF2	BTF3P7, RPI1-80N2.2	
DNAH7	MAML3, BC040304	

ZMYND10	MICA, HLA-B
	SDK1, DL490859
	CA10
	LINC01422, RP1-90L6.2
	FOXP1
	RAB5B, SUOX, CDK2

Table 4: HLA-A interacting proteins extracted from innate DB

Interactors	**Fullname**	**Species**	**Interaction Type**	**Interactor type**	**Supporting Publications**	**Source Database ID(s)**
KIR3DL1 :: HLA-A	Complex of 3 interactors	Homo sapiens	physical association	protein - protein	1	IDB-331164;
LILRB2 :: HLA-A :: HLA-B :: HLA-C	Complex of 4 interactors	Homo sapiens	physical association	protein - protein	1	IDB-1631962;
HLA-A :: TAP1	HLA-A interacts with TAP1	Homo sapiens	physical association	protein - protein	2	BIOGRID-656120; IDB-967886; BIOGRID-305565; IDB-967873; IDB-967877; IDB-967884; BIOGRID-305570;
LILRB2 :: HLA-A	LILRB2 interacts with HLA-A	Homo sapiens	physical association	protein - protein	1	IDB-120685;

LILRB1 :: HLA-A	LILRB1 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	IDB-120686;
MEX3C :: HLA-A	MEX3C interacts with HLA-A	Homo sapiens	transcriptional regulation	protein rna	-	1	IDB-649185;
HLA-A :: KIR2DS4	Complex of 3 interactors	Homo sapiens	physical interaction	protein protein	-	1	IDB-224469; IDB-331173;
TRAC :: HLA-A	TRAC interacts with HLA-A	Homo sapiens	association	protein protein	-	1	BIOGRID-289251;
MAGEA4 :: HLA-A	MAGEA4 physically interacts with HLA-A	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-722358;
HLA-A :: BAG6	HLA-A interacts with BAG6	Homo sapiens	physical association	protein protein	-	1	BIOGRID-935933; BIOGRID-935934;

LILRB1 :: HLA-A	LILRB1 interacts with HLA-A	Homo sapiens	association	protein protein	-	1	BIOGRID-255783;
VCP :: HLA-A	VCP interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-936213;
HLA-A :: MAGEA4	HLA-A interacts with MAGEA4	Homo sapiens	association	protein protein	-	1	BIOGRID-280213;
CD8A :: HLA-A	CD8A physically interacts with HLA-A	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-276690;

UBC :: HLA-A	UBC interacts with HLA-A	Homo sapiens	physical association	protein protein	-	17	BIOGRID-943682; BIOGRID-560951; BIOGRID-593371; BIOGRID-845127; BIOGRID-519106; BIOGRID-731192; BIOGRID-939809; BIOGRID-1030815; BIOGRID-608885; BIOGRID-848071; BIOGRID-695391; BIOGRID-985900; BIOGRID-935893; BIOGRID-628933; BIOGRID-946829; BIOGRID-609072; BIOGRID-619617;

SUMO1 :: HLA-A	SUMO1 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-940712;
B2M :: HLA-A	B2M physically interacts with HLA-A	Homo sapiens	physical interaction	protein protein	-	3	BIOGRID-797898; BIND-330499; BIND-330029;
HLA-A :: LMAN1	Colocalization of HLA-A and LMAN1	Homo sapiens	colocalization	protein protein	-	1	BIOGRID-722349;
NEDD8 :: HLA-A	NEDD8 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-940322;
HLA-A :: TAP2	HLA-A interacts with TAP2	Homo sapiens	physical association	protein protein	-	1	BIOGRID-797903;
HLA-A :: BCAP31	HLA-A physically interacts with BCAP31	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-722342;

COPB1 :: HLA-A	COPB1 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-305567;
JUN :: HLA-A	JUN interacts with HLA-A	Homo sapiens	physical association	protein dna	-	1	BIND-301498;
TAPBP :: HLA-A	TAPBP interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-305569; BIOGRID-305561;
COPG :: HLA-A	COPG interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-305568;
HLA-A :: ZDHHC17	HLA-A interacts with ZDHHC17	Homo sapiens	physical association	protein protein	-	1	EBI-9090133;
HLA-A :: UBC	HLA-A physically interacts with UBC	Homo sapiens	physical interaction	protein protein	-	2	BIOGRID-942127; BIOGRID-608886;

HLA-A :: STAT3	HLA-A physically interacts with STAT3	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-892588;
HLA-A :: GNAS	HLA-A interacts with GNAS	Homo sapiens	physical association	protein protein	-	1	BIOGRID-905779;
ATM :: HLA-A	ATM interacts with HLA-A	Homo sapiens	association	protein protein	-	1	BIOGRID-860011;
GNAS :: HLA-A	GNAS interacts with HLA-A	Homo sapiens	association	protein protein	-	1	BIOGRID-905778;
HLA-A :: MAGEA1	HLA-A physically interacts with MAGEA1	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-283117;

SYVN1 :: HLA-A	SYVN1 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-593369;
HLA-A :: TRAM1	HLA-A interacts with TRAM1	Homo sapiens	physical association	protein protein	-	1	BIOGRID-685381;
UBE2J1 :: HLA-A	UBE2J1 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-593375;
B2M :: HLA-A	B2M interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-797897;
HLA-A :: SEC61A1	HLA-A interacts with SEC61A1	Homo sapiens	physical association	protein protein	-	1	BIOGRID-695392;
CALR :: HLA-A	CALR physically interacts with HLA-A	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-856544;

HLA-A :: KIR3DL2	HLA-A physically interacts with KIR3DL2	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-288301;
BCAP31 :: HLA-A	BCAP31 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-724004;
CD8A :: HLA-A	CD8A interacts with HLA-A	Homo sapiens	association	protein protein	-	1	BIOGRID-275790;
UGGT1 :: HLA-A	UGGT1 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-242729;
PSMD2 :: HLA-A	PSMD2 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-593377;

HLA-A :: MEOX2	HLA-A physically interacts with MEOX2	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-951350;
MMS19 :: HLA-A	MMS19 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	1	BIOGRID-856347;
DERL1 :: HLA-A	DERL1 interacts with HLA-A	Homo sapiens	physical association	protein protein	-	2	BIOGRID-593376; BIOGRID-251198;

Table 5: HLA-A interacting proteins extracted from innate DB

Interactors	**Fullname**	**Species**	**Interaction Type**	**Interactor Types**	**Supporting Publications**	**Source Database ID(s)**
LILRB2 :: HLA-A :: HLA-B :: HLA-C	Complex of 4 interactors	Homo sapiens	physical association	protein - protein	1	IDB-1631962;
HLA-B :: KIR2DL3	Complex of 3 interactors	Homo sapiens	physical interaction	protein - protein	1	IDB-224467; IDB-331171;
HLA-B :: UBD	HLA-B interacts with UBD	Homo sapiens	physical association	protein - protein	1	BIOGRID-733698;
HLA-B :: HSPA5	HLA-B interacts with HSPA5	Homo sapiens	physical association	protein - protein	1	BIOGRID-918202;
VCP :: HLA-B	VCP interacts with HLA-B	Homo sapiens	physical association	protein - protein	1	BIOGRID-241547;

LILRB1 :: HLA-B	LILRB1 physically interacts with HLA-B	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-256234;
SUMO1 :: HLA-B	SUMO1 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-940433;
TRIM28 :: HLA-B	TRIM28 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-243163;
HLA-B :: PCK1	HLA-B interacts with PCK1	Homo sapiens	physical association	protein protein	-	1	BIOGRID-609514;
NEDD8 :: HLA-B	NEDD8 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-940003;

LILRB2 :: HLA-B	LILRB2 physically interacts with HLA-B	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-256231;
EZR :: HLA-B	EZR interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-242029;
AHCY :: HLA-B	AHCY interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-241853;
NDUFA9 :: HLA-B	Colocalization of NDUFA9 and HLA-B	Homo sapiens	colocalization	protein protein	-	1	BIOGRID-749917;
VARS :: HLA-B	VARS interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-241861;
ADRB2 :: HLA-B	ADRB2 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-875968;

HLA-B :: ARHGEF4	HLA-B physically interacts with ARHGEF4	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-464462;
HLA-B :: HNF4A	HLA-B interacts with HNF4A	Homo sapiens	physical association	protein dna	-	1	BIND-124619;
EDEM1 :: HLA-B	EDEM1 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-1031949;
CD8A :: HLA-B	CD8A physically interacts with HLA-B	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-276693;
HLA-B	HLA-B physically associates with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-1031948;

PSMD1 :: HLA-B	PSMD1 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-242918;
PAICS :: HLA-B	PAICS interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-242651;
ANTXR2 :: HLA-B	ANTXR2 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	EBI-2812057;
UBC :: HLA-B	UBC interacts with HLA-B	Homo sapiens	physical association	protein protein	-	6	BIOGRID-939589; BIOGRID-626060; BIOGRID-628932; BIOGRID-1031307; BIOGRID-731193; BIOGRID-943928;
B2M :: HLA-B	B2M interacts with HLA-B	Homo sapiens	association	protein protein	-	1	BIOGRID-689302;

HLA-B :: UBC	HLA-B physically interacts with UBC	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-942128;
HLA-B :: STAT1	HLA-B physically interacts with STAT1	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-892662;
HLA-B :: TRIB3	HLA-B physically interacts with TRIB3	Homo sapiens	physical interaction	protein protein	-	1	BIOGRID-330260;
TARS :: HLA-B	TARS interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-242069;
PFAS :: HLA-B	PFAS interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-241511;
PLS3 :: HLA-B	PLS3 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-242213;

MSN :: HLA-B	MSN interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-243199;
RUVBL1 :: HLA-B	RUVBL1 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-242897;
MMS19 :: HLA-B	MMS19 interacts with HLA-B	Homo sapiens	physical association	protein protein	-	1	BIOGRID-856348;

Table 6: HLA-A interacting genes with>0.63 scoring from HIPPIE Database

Interactor-UniProt ID	**Interactor - Entrez Gene ID**	**Interactor-gene Symbol**	**Score**
HLAF_HUMAN	3134	HLA-F	0.72
KI3L2_HUMAN	3812	KIR3DL2	0.72
KI3S1_HUMAN	3813	KIR3DS1	0.72
1A66_HUMAN	3105	HLA-A	0.63
BUD31_HUMAN	8896	KIR3DS1	0.63
ILK_HUMAN	3611	ILK	0.63
PGH2_HUMAN	5743	PTGS2	0.63
STAT3_HUMAN	6774	STAT3	0.63

Table 7: HLA-B interacting genes with >0.63 scoring from HIPPIE Database

Interactor-UniProt ID	**Interactor-Entrez Gene ID**	**Interactor-Gene Symbol**	**Score**
B2MG_HUMAN	567	B2M	0.89
CD8A_HUMAN	925	CD8A	0.85
LIRB1_HUMAN	10859	LILRB1	0.85
LIRB2_HUMAN	10288	LILRB2	0.85
KI3S1_HUMAN	3813	KIR3DS1	0.84
1B07_HUMAN	3106	HLA-B	0.76
1A01_HUMAN	3105	HLA-A	0.73
1C01_HUMAN	3107	HLA-C	0.73
AT12A_HUMAN	479	ATP12A	0.73
BIG1_HUMAN	10565	ARFGEF1	0.73
BIG2_HUMAN	10564	ARFGEF2	0.73
CA112_HUMAN	55732	C1orf112	0.73
CLH2_HUMAN	8218	CLTCL1	0.73
CNDH2_HUMAN	29781	NCAPH2	0.73
ERAP1_HUMAN	51752	ERAP1	0.73
F91A1_HUMAN	157769	FAM91A1	0.73

HD_HUMAN	3064	HTT	0.73
HEAT6_HUMAN	63897	HEATR6	0.73
HLAE_HUMAN	3133	HLA-E	0.73
HLAH_HUMAN	3136	HLA-H	0.73
INT4_HUMAN	92105	INTS4	0.73
INT5_HUMAN	80789	INTS5	0.73
LTN1_HUMAN	26046	LTN1	0.73
PDK1_HUMAN	5163	PDK1	0.73
PI4KA_HUMAN	5297	PI4KA	0.73
SGSM3_HUMAN	27352	SGSM3	0.73
SIAE_HUMAN	54414	SIAE	0.73
TTC28_HUMAN	23331	TTC28	0.73
WDFY3_HUMAN	23001	WDFY3	0.73
EZRI_HUMAN	7430	EZR	0.72
KI3L2_HUMAN	3812	KIR3DL2	0.72
MOES_HUMAN	4478	MSN	0.72
PLST_HUMAN	5358	PLS3	0.72
PSMD1_HUMAN	5707	PSMD1	0.72
PUR4_HUMAN	5198	PFAS	0.72
PUR6_HUMAN	10606	PAICS	0.72
RUVB1_HUMAN	8607	RUVBL1	0.72
SAHH_HUMAN	191	AHCY	0.72
SYTC_HUMAN	6897	TARS	0.72
SYVC_HUMAN	7407	VARS	0.72
TERA_HUMAN	7415	VCP	0.72
TIF1B_HUMAN	10155	TRIM28	0.72
	117189	GRDX	0.63
1C04_HUMAN	3107	HLA-C	0.63
ACCN4_HUMAN	55515	ASIC4	0.63
ADA21_HUMAN	8747	ADAM21	0.63
ADRB2_HUMAN	154	ADRB2	0.63

AKT2_HUMAN	208	AKT2	0.63
ARHG4_HUMAN	50649	ARHGEF4	0.63
AT1A3_HUMAN	478	ATP1A3	0.63
CALR_HUMAN	811	CALR	0.63
CC50A_HUMAN	55754	TMEM30A	0.63
CXCR4_HUMAN	7852	CXCR4	0.63
DHSA_HUMAN	6389	SDHA	0.63
EDEM1_HUMAN	9695	EDEM1	0.63
EGFR_HUMAN	1956	EGFR	0.63
EGLN3_HUMAN	112399	EGLN3	0.63
ERBB3_HUMAN	2065	ERBB3	0.63
FAF2_HUMAN	23197	FAF2	0.63
FCGRN_HUMAN	2217	FCGRT	0.63
FLNB_HUMAN	2317	FLNB	0.63
GBRAP_HUMAN	11337	GABARAP	0.63
GCP2_HUMAN	10844	TUBGCP2	0.63
GRP78_HUMAN	3309	HSPA5	0.63
HEAT1_HUMAN	55127	HEATR1	0.63
HLAG_HUMAN	3135	HLA-G	0.63
IPO13_HUMAN	9670	IPO13	0.63
MCAF1_HUMAN	55729	ATF7IP	0.63
MMS19_HUMAN	64210	MMS19	0.63
NEDD8_HUMAN	4738	NEDD8	0.63
NEK4_HUMAN	6787	NEK4	0.63
PCKGC_HUMAN	5105	PCK1	0.63
PDIA3_HUMAN	2923	PDIA3	0.63
PP16A_HUMAN	84988	PPP1R16A	0.63
PTPRO_HUMAN	5800	PTPRO	0.63
RAB10_HUMAN	10890	RAB10	0.63
RIPK4_HUMAN	54101	RIPK4	0.63
RNF4_HUMAN	6047	RNF4	0.63

S39A3_HUMAN	29985	SLC39A3	0.63
STAT1_HUMAN	6772	STAT1	0.63
SUMO1_HUMAN	7341	SUMO1	0.63
TAP1_HUMAN	6890	TAP1	0.63
TBA4A_HUMAN	7277	TUBA4A	0.63
TECT2_HUMAN	79867	TCTN2	0.63
TM11B_HUMAN	132724	TMPRSS11B	0.63
TPSNR_HUMAN	55080	TAPBPL	0.63
TRIB3_HUMAN	57761	TRIB3	0.63
UBD_HUMAN	10537	UBD	0.63
UXS1_HUMAN	80146	UXS1	0.63
WDR11_HUMAN	55717	WDR11	0.63