T.C.
ÇUKUROVA UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF TRANSLATIONAL MEDICINE

THE IMPACT OF EARLY LIFE STRESS ON AIRWAY REMODELING AND SEVERE ASTHMA RELATED GENE PROFILE IN MICE

SEHREEN TORY

TRANSLATIONAL MEDICINE MASTER'S PROGRAM (WITH THESIS)
MASTER'S THESIS

ADVISOR
Assoc. Prof. Dr. Yasemin SAYGIDEGER

SECOND ADVISOR
Asst. Prof. Dr. Keziban Korkmaz BAYRAM

ADANA-2024

T.C.
ÇUKUROVA UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF TRANSLATIONAL MEDICINE

THE IMPACT OF EARLY LIFE STRESS ON AIRWAY REMODELING AND SEVERE ASTHMA RELATED GENE PROFILE IN MICE

SEHREEN TORY

TRANSLATIONAL MEDICINE MASTER'S PROGRAM (WITH THESIS)
MASTER'S THESIS

ADVISOR
Assoc. Prof. Dr. Yasemin SAYGIDEGER

SECOND ADVISOR
Asst. Prof. Dr. Keziban Korkmaz BAYRAM

This study was supported by the Scientific Research Projects Unit at Çukurova University (Project Number: TYL-2023-15988)

ADANA-2024

***I dedicate this thesis to my parents**, who have been my constant source of inspiration and support throughout this journey. Their unwaiving belief in me has been instrumental in bringing me to this point.*

***And to all my teachers**,*

whose guidance and mentorship have paved the way for my success.

ACKNOWLEDGEMENT

I would like to express my deep gratitude to my supervisor, Assoc. Prof. Dr. Yasemin Saygideger. She has been incredibly supportive and has made my journey much smoother. Her presence has consistently motivated me to generate novel ideas.

I am extremely grateful for the guidance and support provided by my second advisor, Asst. Prof. Dr. Keziban Korkmaz Bayram, throughout my thesis period.

I have the utmost respect for Prof. Dr. Behice Durgun, who is in charge of the Translational Medicine department. I eagerly anticipate any chance to engage in conversation with her, as it allows me to gain further insights.

I would like to express sincere thanks to Prof. Dr. Mehmet Bertan Yilmaz and Asst. Prof. Yusuf Kemal Arslan for their invaluable support.

I would like to thank all of my lab-mates from Saygideger lab and Merlab (Central research laboratory application and research center, AYBU).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ASM	Airway smooth muscle
ASMC	Airway smooth muscle cells
Beas 2B	Human bronchial epithelial cell line
CCL11	C-C motif chemokine ligands CCL11
CHI3L1	Chitinase 3 like 1
CRF	Corticotropin-releasing factor
CRP	C-reactive protein
ECM	Extracellular matrix
ELS	Early life stress
EpC	Bronchial epithelial cells
GC	Glucocorticoid
IL-33	Interleukin-33
MMP-9	Matrix metalloprotenase-9
MS	Maternal Separation
POSTN	Periostin
TGF-β1	Transforming growth factor beta 1
Th1	T Helper cell 1
Th17	T Helper cell 17
Th2	T Helper cell 2
TNF-α	Tumor necrotic factor-alpha

ABSTRACT

The Impact of Early Life Stress on Airway Remodeling And Severe Asthma Related Gene Profile in Mice

Aim and objective: Neonatal and infants may suffer from stress due to many reasons. Stress may be unavoidable nowadays and has a negative impact which may be the initiator of different diseases. Early life stress influences the airway, which may result in asthma at any stage of life; it also impacts the severity of asthma and the airway structure. Chronic stress increases cortisol levels via Hypothalamic-pituitary-adrenal (HPA) axis activation. Imbalanced cortisol increases the pro-inflammatory cytokines in the blood, which induce inflammation. In this study, mRNA expression of the airway remodeling and severe asthma-related genes were investigated in the early-life stressed mice and in vitro chronic low and high-stress mimic conditions by administrating dexamethasone.

Methods: In this study, mRNA expression of the airway remodeling and severe asthma-related genes were investigated in the early-life stressed mice and in vitro chronic low and high-stress mimic conditions. The mice were separated the mother 3 hours daily from postnatal days 1-14 (PND1-PND14), and blood was collected on PND35. The administration of chronic low (10nM) and chronic high (100nM) of dexamethasone for a duration of five days in vitro Beas 2B cell to produce chronic low and chronic high stress mimic condition. . RNA was extracted from the blood of mice and human bronchial epithelial cell Beas 2B followed by the synthesis of complementary DNA (cDNA). RT-PCR was performed to evaluate the mRNA expression level. Graphical presentation was performed in Graph pad prism 8.4.3.

Results: In vivo results shows that early life stress has an elevation of the *Postn, Chi3l1 & Ccl11* genes, which are related to asthma severity and airway remodeling. In vitro, chronic low and high stress has been found to have an impact on the expression of *IL-33* levels by increasing significantly (fold change 4.9 and 3.28 respectively).

Conclusion: Exposure to stress during early life has been found to have a significant impact on the development of asthma and the remodeling of airways.

Keywords: Early life stress, Chronic Stress, Airway remodeling, Severe asthma, dexamethasone.

ÖZET

Farelerde Erken Yaşam Stresinin Havayolu Yeniden Şekillenmesi ve Şiddetli Astıma İlişkin Gen İfade Profili Üzerindeki Etkisi

Amaç ve hedef: Yenidoğan ve bebekler birçok nedenden dolayı stresten muzdarip olabilirler. Stres günümüzde kaçınılmaz olabilir ve farklı hastalıkların başlatıcısı olabilecek olumsuz bir etkiye sahiptir. Erken yaşam stresi hava yolunu etkiler ve bu da yaşamın herhangi bir aşamasında astıma neden olabilir; Ayrıca astımın şiddetini ve hava yolu yapısını da etkiler. Kronik stres, Hipotalamik-hipofiz-adrenal (HPA) eksen aktivasyonu yoluyla kortizol seviyelerini arttırır. Dengesiz kortizol, kandaki iltihaplanmaya neden olan proinflamatuar sitokinleri artırır. Bu araştırmada, deksametazon uygulanarak erken yaşam stresli farelerde ve in vitro kronik düşük ve yüksek stres indüklenen solunum epitel hücresi modelinde hava yolunun yeniden şekillenmesi ve şiddetli astımla ilişkili genlerin ekspresyon düzeylerinin karşılaştırılması hedeflendi.

Yöntemler: Erken yaşam stres grubu yavrular (n=24), doğum sonrası 1-14 günlerinden (PND1-PND14) günde 3 saat anneden ayrıldı ve kontrol grubu yavrular (n=24) ayrılmadı. PND35'te kan alındı. 10nM ve 100nM deksametazon uygulaması, kronik düşük ve kronik yüksek stres taklit durumu üretmek için beş günlük bir süre boyunca in vitro insan bronşiyal epitel hücresi Beas 2B hücresi olarak yapıldı. RNA, farelerin kanından ve insan bronşiyal epitel hücresi Beas 2B'den ekstrakte edildi ve ardından tamamlayıcı DNA (cDNA) sentezi yapıldı. mRNA ekspresyon seviyesini değerlendirmek için RT-PCR yapıldı. Grafik sunumu Graphpad prizma 8.4.3'te yapıldı.

Sonuçlar: İn vivo sonuçlar, erken yaşam stresinin, astım şiddeti ve hava yolunun yeniden şekillenmesi ile ilişkili olan *Postn*, *Chi3l1* ve *Ccl11* genlerinde bir yükselmeye sahip olduğunu göstermektedir. İn vitro kronik düşük ve yüksek stresin *IL-33* düzeylerinin ekspresyonu üzerinde önemli ölçüde artarak etkili olduğu bulunmuştur (kat değişimi sırasıyla 4.9 ve 3.28).

Sonuç: Kronik strese maruz kalmanın astım gelişimi ve hava yollarının yeniden şekillenmesi üzerinde önemli bir etkisi olduğu bulunmuştur.

Anahtar Kelimeler: **Erken yaşam stresi, Kronik stres, Hava yolu yeniden şekillenmesi, Şiddetli astım, deksametazon.**

1. INTRODUCTION

Asthma is a long-term respiratory condition that can impact individuals of any age. It is characterized by periodic and reversible episodes of wheezing, chest tightness, difficulty breathing, and coughing[1]. As per the ATS/ERS guideline, severe asthma is characterized by the requirement for high dose inhaled corticosteroids (ICS) along with another controller medication (and/or systemic corticosteroid) to manage the condition and prevent it from becoming or remaining "uncontrolled"[2]. Approximately 6% of individuals with asthma experience severe symptoms, which significantly impact their daily lives and increase the likelihood of asthma attacks, hospitalizations, and even mortality[3]. Stress is a common psychophysiological reaction that occurs in the body as a result of unfavorable, demanding, and challenging events or stressors[4,5]. The body contains several systems that independently or jointly manage stress levels [6,7], such as the hypothalamic-pituitary-adrenal axis (HPA-Axis), autonomic nervous system (ANS), and immunological system [8,9,10,11]. Stress can last for either an extended duration or a brief duration. Stress can be classified into two categories: acute and chronic. Acute stress is a temporary condition that often lasts for a brief duration, typically ranging from minutes to hours. Chronic stress, in contrast, has a prolonged duration, ranging from days to weeks or even months [12]. Chronic stress leads to the release of specific hormones or chemicals from the body, indicating a continuous state of stress and impacting crucial organs like the brain, heart, and liver in ways that may be detrimental to one's health [13]. Cortisol primarily regulates psychological stress, with the HPA axis serving as the principal mechanism that responds to psychosocial stress and interacts with the autonomic nervous system (ANS) and the immune system [6,7]. Adverse early life and chronic stress disrupts the normal functioning of the HPA axis [14], resulting in an elevated release of pro-inflammatory cytokines. Studies show that there is a continuous and long-lasting inflammation in the airways, which is marked by high levels of proinflammatory cytokines (IL-1β, IL-4, IL-5, IL-6, IL-13, and TNF-α), reduced levels of anti-inflammatory cytokines (IL-1ra and IL-10), and an increase in the infiltration of inflammatory cells [15]. A meta-analysis found that animals experiencing early life stress (ELS) have increased levels of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, but anti-inflammatory IL-10 did not change [16]. A meta-analysis by Baumeister et al. Found

that persons with a history of childhood trauma had higher levels of CRP, IL-6, and TNF-α in adulthood [17]. The impact of the pro-inflammatory cytokine, TNF-α, causes the release of periostin, a protein implicated in the inflammation and restructuring of the airways in type-2 airway inflammation [18] and upregulation of C-C motif chemokine ligands CCL11 [19]. Some genes, such as *POSTN* [20], *CCL11*[21], *CHI3L1*[22], *IL-33*[23],*TGF-ß1* [24], *MMP9* are found to be upregulated and association with severe asthma. In this article, we experimented whether early life stress causes *Postn*, *Ccl11* and *Chi3l1* upregulation or not in mice blood. And chronic low or high stress mimicking dexamethasone concentrations *POSTN*, *CCL11*, *CHI3L1*, *IL-33, TGF-ß1* and *MMP9* expression in human normal broncial epithelial cell Beas 2B cell line.

2. GENERAL INFORMATION

2.1. Asthma and severe asthma:

Asthma is a persistent respiratory ailment that impacts the air passages. The condition is characterized by reversible airflow obstruction and airway hyperresponsiveness, inflammation increased expression of numerous cytokines, chemokines, and other inflammatory mediators which possibly contribute to infiltrating inflammatory cells and remodeling in the airways and constriction of the air passages, leading to breathing difficulties and decreased airflow[25,26](Yang et al., 2017). Although there have been significant improvements in treatment, asthma continues to result in 420,000 fatalities annually and impacts 300 million individuals worldwide[27]. The World Health Organization (WHO) established a definition for severe asthma in 2009. It refers to asthma that is not under control and poses a high risk of frequent severe exacerbations, potential death, adverse reactions to medications, and chronic morbidity. Severe asthma is further classified into three groups: untreated severe asthma, difficult-to-treat severe asthma, and treatment-resistant severe asthma[28]. The proportion of individuals with severe asthma is estimated to range from 5% to 10% of the total asthmatic population[29,30,31]. Severe asthmatic individuals accounted for 17% of asthma deaths, according to the UK National Review of Asthma Deaths[32].

2.2. Airway remodeling and severe asthma

Airway remodeling is the most significant pathophysiologic feature of asthma. It refers to the alteration of the respiratory passages due to structural modifications, including changes such as the transformation of goblet cells, the accumulation of proteins, and the development of fibrosis in the underlying tissue. Additionally, it involves the excessive production of substances that promote bloodvessel growth and the enlargement and multiplication of smooth muscle cells in the airway walls[33]. Airway remodeling with progressive alterations in the epithelial layer of the respiratory tract is a severe consequence of asthma[34].

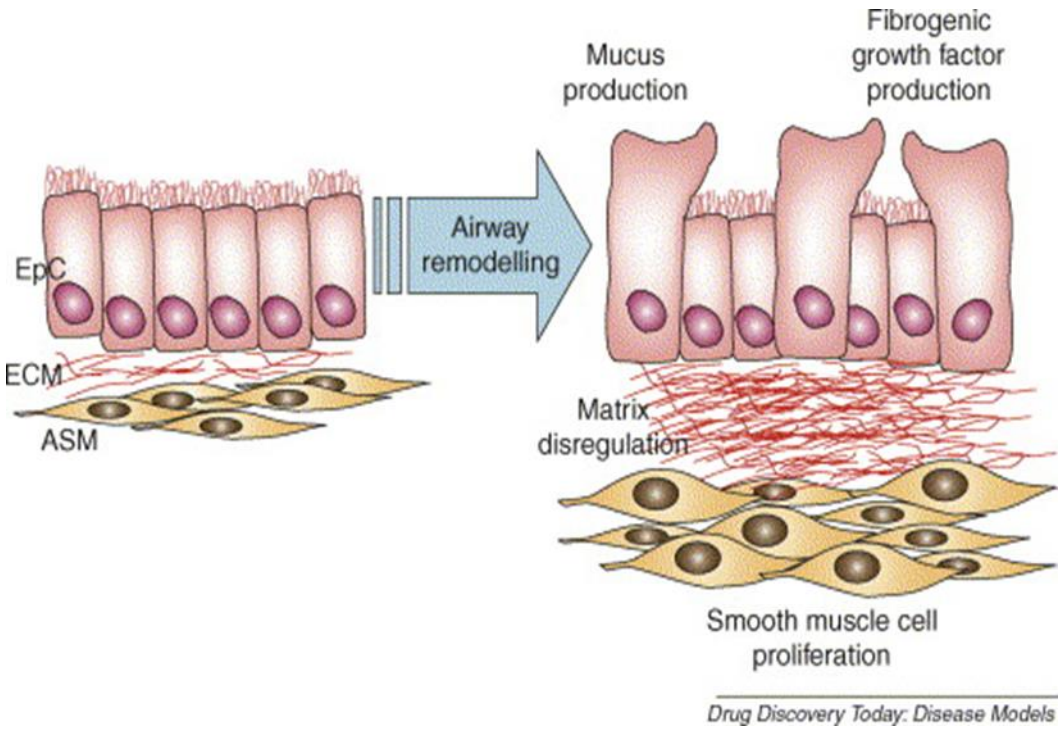

Figure 2.1. Airway remodeling[35]

The structural changes involve hyperplasia of the bronchial epithelial cells (EpC), resulting in excessive mucus secretion and potential airway blockage. An alteration in the metabolism of extracellular matrix (ECM), characterized by either an excessive production or a diminished breakdown of matrix proteins, results in the accumulation of collagens and other matrix proteins in the subepithelial and submucosal regions. Hyperplasia of airway smooth muscle (ASM) and proliferation of myofibroblasts contribute to the increased myocyte muscle mass. The convergence of these processes results in constriction of the airways, consequently leading to diminished pulmonary function[35] (Figure 2.1)

Upstream cytokines generated by bronchial epithelial cells are responsible for initiating immunologic processes that ultimately result in airway remodeling. The latter is a complicated process that calls for the precise expression of fibrogenic and angiogenic factors at the appropriate moment. These factors produce significant structural changes in the bronchial walls and blood vessels. These modifications contribute to a reduction in airway diameter and stiffness of the airways, which results in restrictions in airflow and respiratory discomfort[33].

Increased airway smooth muscle (ASM) mass is a significant factor in airway remodeling. This increase is believed to be caused by the migration of ASM cells, contributing to the local cellular hyperplasia. The origin of these airway smooth muscle cells (ASMCs) has been attributed to the infiltration of nearby myofibroblasts, adjacent ASMCs, and circulating hematopoietic progenitor cells (HPCs)[36].

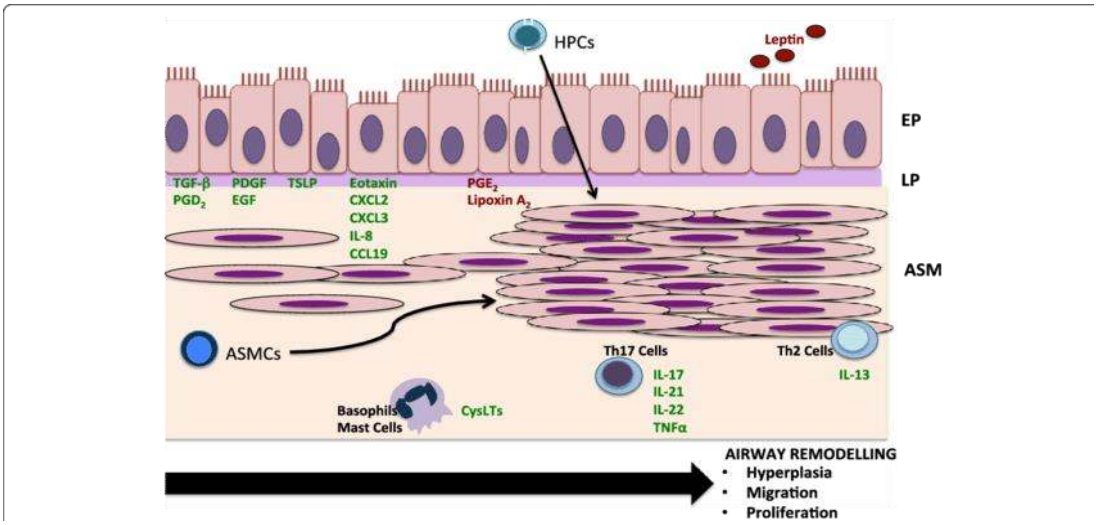

Figure 2.2. Mediators of airway remodeling[36]

Figure 2.2 outlines various mediators that have been identified to influence the migration of airway smooth muscle cells (ASMC). The airway epithelial cells produce local pro-inflammatory mediators such as TGF-β, PDGF, EGF, PGD2, CXCL2, CXCL3, IL-8, eotaxin, TSLP, and CCL19. These mediators have been demonstrated to stimulate the migration of airway smooth muscle cells (ASMC). Moreover, the migration of airway smooth muscle cells (ASMC) is additionally influenced by the production of inflammatory cytokines by Th17 and Th2 cells, as well as the production of CysLTs by basophils and mast cells. On the other hand, PGE2, lipoxin A2, leptin act as inhibitor of this process[36].

The presence of structural airway alterations is evident in asthma, even in cases of mild disease. However, the extent of remodeling tends to worsen as the severity of the disease increases. Furthermore, the pathological changes begin at an early, specifically during the preschool years, and they become firmly established by the time the individual

reaches school age. These changes persist throughout adulthood, even though symptoms may temporarily improve during certain periods[37].

2.3. Stress and its effect:

Stress is the body's general and non-specific reaction to a possible disruption of its internal balance, caused by any change in the environment, whether internal or external. This response is characterized by various behavioural and physical reactions. The primary objective of all those actions and reactions is to adapt to the stressor. The reaction to such a stressor exhibits significant variability and is contingent upon genetic factors and coping mechanisms unique to each individual[38]. Stress impacts the body and is strongly associated with inflammation, triggering inflammatory pathways in both the brain and peripheral tissues. Prolonged stress can lead to enduring physiological and psychological alterations. Stress-induced inflammation is a significant contributing factor in the onset of numerous inflammatory disorders[39].

2.4 Chronic stress and HPA axis:

HPA axis is a significant contributor to the stress response. It consists of various components within the central nervous system and peripheral tissues. The primary actors are situated in the paraventricular nucleus of the hypothalamus (PVN), the anterior lobe of the pituitary gland, and the adrenal gland. Neurons in the PVN produce and release corticotropin-releasing factor (CRF), which plays a crucial role in regulating the HPA axis[8].

CRF stimulates the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH) into the bloodstream in response to a stressor. The circulating ACTH specifically acts on the adrenal cortex, prompting the production and release of glucocorticoids (GCs), such as cortisol (figure 2.3). This process regulates the stress response in peripheral tissues[40;41].

Glucocorticoids are hormones that control the physiological changes and subsequent effects of the HPA axis in reaction to stress[41]. Glucocorticoids can exert either a pro-inflammatory or anti-inflammatory impact on the immune system, based on the

prevailing circumstances[39]. Typically, GC secretions adhere to a circadian rhythm. However, a stressful event may replace this rhythm and result in an immediate surge in GC levels[41].

Cortisol, a significant glucocorticoid, is released in response to both acute and chronic stress[39]. Typically, cortisol exhibits a diurnal rhythm characterized by an elevation during the night prior to awakening, a brief surge after waking up, and a gradual decrease until bedtime. Cortisol regulates the body's response to stress[41].

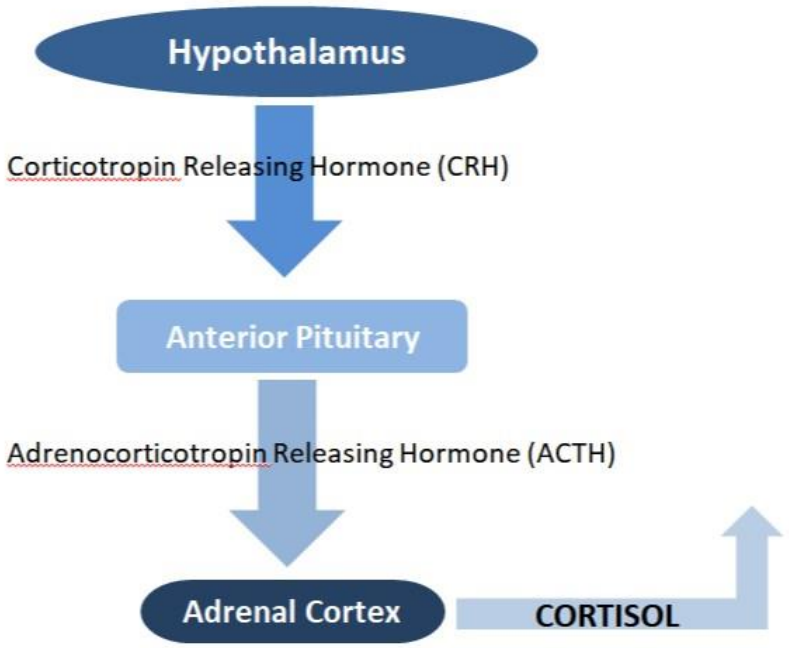

Figure 2.3. Hypothalamic-Pituitary-Adrenal axis

Persistent stimulation of the HPA axis has been demonstrated to alter the immune response by favoring a shift from a predominantly T-helper 1 cellular response to a T-helper 2 response phenotype. This shift leads to an augmentation in the production of inflammatory cells and the occurrence of atopic inflammation. The changes in immune response caused by exposure to stress have been postulated to contribute to the progression of atopic march and inflammation, potentially leading to the onset of asthma[42] (Figure 2.4).

On the otherhand, chronic stress causes alteration of beta-adrenergic (B2AR) and the glucocorticoid receptor (GR-α) in children via HPA axis with is associated with asthma severity[42]. Children with asthma who experienced acute on chronic stress exhibited 9.5- and 5.5-fold changes in mRNA expression of B2AR and GR-α, respectively[43], which is assocoiated with asthma severity.

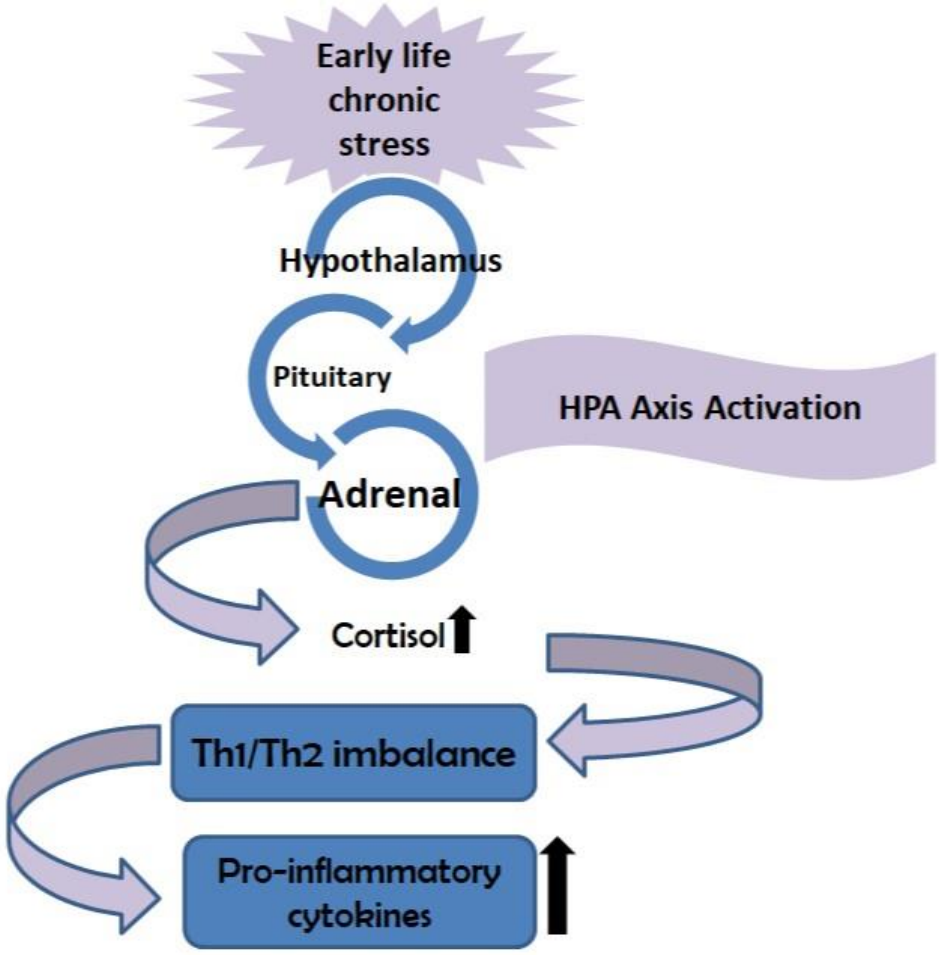

Figure 2.4. Chronic stress and inflammatory response

2.5. Early life stress and inflammation

Early-life stress can cause enduring alterations in the HPA axis of a child, leading to persistent elevations in circulating corticosteroid levels[44]. Due to the inhibitory effects of increased corticosteroid levels, newborns who experience stress in early life can show reduced body weights[44,45,46,] which result in lungs that are not fully developed and incomplete separation of the alveoli. Furthermore, it has been observed that early

exposure to stress leads to heightened sensitivity to prolonged stress in later stages of life[44] and individuals with asthma may experience increased airway inflammation. Studies has demonstrated that neonatal psychological stress can lead to lasting physiological, psychological, and neurological consequences[47,48,49,50].

A meta-analysis performed by Baumeister et al. demonstrated that individuals with a background of childhood trauma consistently displayed an increase in C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) during adulthood[17]. Another meta-analysis demonstrated that animals subjected to early life stress (ELS) exhibit an elevation in pro-inflammatory cytokines IL-1β, IL-6, and TNF-α. However, there was no change observed in the anti-inflammatory cytokine IL-10[16].

2.6 Early life stress and asthma:

The early stages of life are crucial for growth and development. Stress during this period can have long-lasting effects on various organs, including the brain, lungs, gastrointestinal tract, and immune system. This can not only contribute to conditions such as autism, depression, lung tissue abnormalities, and irritable bowel syndrome[51,52,53] , but also worsen symptoms of asthma[54,55,56] (Table 2.1).

Table 2.1. Early life stress, adverse childhood effects or toxic stress and asthma association:

Aim of the study	Outcome age group	Conclusion	Reference
To determine if within-day variability in stress is associated with increased asthma symptomatology	Adolescents	Experiences of stress associated with increased shortness of breath, self-report of wheezing	57
To determine the interaction effect of community violence exposure, protective factors, and asthma morbidity	Children	Higher caregiver community violence exposure predicted increased asthma health care utilization	58
To examine differences in asthma outcomes by levels of child reported neighborhood and family stress	Children	Increased neighborhood and family stress was associated with poorly controlled asthma	59

To determine if a cumulative risk index including psychosocial stress exposures is associated with asthma morbidity	Children	Higher cumulative risk index was associated with increased asthma related functional limitation, hospitalizations, and ED visits	[60]
To determine the association between prenatal stress and occurrence of atopic dermatitis, food allergy, wheezing, recurrent respiratory tract infections	Children	Association between perceived maternal stress exposure and risk of infant wheezing in first year of life	[61]
To determine the relationship between whether women's experiences of common adverse experiences during pregnancy was associated with the risk of developing atopic disease in the child	Children	Prevalence of asthma at age 14 years in the child was associated with increased prenatal stress exposure	[62]
To determine the association between ACEs and adult health and impact of socioeconomic status	Adult	High Conventional ACEs increases the odds ratio for asthma	[63]
To determine the association between childhood trauma and adulthood asthma	Adult	Early life adversity induces epigenetic modifications in DNA, can potentially contribute to the onset and progression of asthma.	[64]

2.7. Early life chronic stress and airway remodeling:

Early life chronic stress causes HPA axis stimulation which results in increased glucocorticoid secretion and dysregulation of natural secretion. This neuroendocrine response enhances Th2-type immune responses in the lungs by causing an imbalance between Th1/Th2 or regulatory T cell/Th2. This response is closely linked to the exacerbation of asthma caused by psychological stress and glucocorticoid resistance. Th2-associated cytokines, such as interleukin-4, -5, -13, -25, and -33, play a role in airway inflammation, hyperresponsiveness, and remodeling. These processes contribute to a gradual deterioration of lung function. A study has shown that the chronic inflammation of the airways is sustained and characterized by high levels of proinflammatory cytokines

(IL-1β, IL-4, IL-5, IL-6, IL-13, and TNF-α), reduced levels of anti-inflammatory cytokines (IL-1ra and IL-10), and intense infiltration of inflammatory cells in the airways[15].

Cells such as T helper (Th) cells, eosinophils, neutrophils, and mast cells enter the airway and interact with resident cells like fibroblasts, smooth muscle cells, neuronal cells, epithelial cells, and endothelial cells. This interaction involves the release of various cytokines, enzymes, metabolites, and growth factors. Over time, this signaling environment leads to changes in the structure of the airways, known as airway remodeling, particularly in chronic conditions. Again, T helper cells, particularly Th2 cells, respond by releasing a distinct set of cytokines, such as IL-4, IL-5, IL-9, and IL-13. When the cytokines IL-4, IL-5, IL-9, IL-11, or IL-13 are overexpressed, individuals experience the spontaneous development of airway inflammation, increased airway hyperresponsiveness (AHR), excessive production of mucus, and structural changes in the airways[65].

2.8. Some airway remodeling and severe asthma associated genes

Periostin is a matricellular protein encoded by the *POSTN* gene in humans[66]. *POSTN* is one of the genes most increased in airway epithelial cells from people with asthma[67]. Periostin serves as an indicator for diseases mediated by Th2 cytokines. Periostin is involved in type-2 airway inflammation and remodeling. Pro- inflammatory cytokines, tumor necrosis factor-α (TNF-α), can influence *POSTN* secretion[18]. The periostin level in the serum is thought to reflect its production level in the airways of people with asthma, and it does not change much over time. A meta-analysis compared the levels of periostin in the serum of people with asthma and healthy people, and the result was that the levels of periostin in the serum were significantly higher in adults with asthma than in healthy people[20].

CCL11, or eotaxin-1, is a chemokine involved in immune responses and inflammation. TNF-α induces the upregulation of C-C motif chemokine ligands CCL11[19]. *CCL11* plays an indirect effect in airway remodeling through the recruitment of eosinophils and mast cells, both of which exhibit activities related to profibrogenesis[68].

The migration of smooth muscle cells in the airways is mediated by CCL11[69] contribute to airway remodeling triggered by chronic inflammation[70].

YKL-40, a non-enzymatic protein encoded by the CHI3L1 gene, is involved in asthma pathogenesis. It can bind to chitin and is associated with airway remodelling and inflammation, impairing lung function and disease progression. The pro-inflammatory cytokine TNF-α induce CHI3LI in alveolar macrophages, chondrocytes, colonic epithelial cells, and various cancer cells[71]. Elevated Chi3l1 causes bronchial smooth muscle cell proliferation and migration which leads to airway remodeling[72]. The serum YKL-40 level is elevated in immune diseases, cancers, and infections. A meta-analysis revealed that asthmatic patients have significantly higher levels of YKL-40 compared to healthy individuals, and its levels increase with the severity of asthma[22]. Konradsen et al. conducted a study to compare the levels of YKL-40 in the serum samples of children with therapy-resistant asthma (n=34), controlled persistent asthma (n=36), and healthy control (n=27). Children with therapy-resistant asthma exhibited elevated levels of serum YKL-40, which were strongly associated with increased airway thickness and asthma control[73]. Another study found that YKL-40 levels in serum were correlated with the thickness of airway subepithelial basement membrane[74].

A member of the IL-1 cytokine family, IL-33, is released to alert immune cells that express the IL-1 receptor like-1 (IL-1R1 or ST2) during cell damage or tissue injury. Human IL-33 gene is mainly expressed by structural cells of various tissues, including fibroblasts, airway smooth muscle cells (ASMC), and endothelial and epithelial cells. IL-33, a bronchial epithelial cell-derived alarmin, is an upstream cytokine that initiate immunologic events culminating in airway remodeling[33]. A recent meta-analysis suggests a strong link between IL-33 levels and the degree to which asthma symptoms were manifested. The level of IL-33 that may be measured in serum or plasma has the potential to serve as a valuable biomarker of asthma or the severity of the condition[23]. Antigen-induced asthmatic responses are made worse by IL-33, which causes airway hyperresponsiveness, inflammation and remodeling, mast cell activation, and synthesis of IgE and cytokines[75].

TGF-β is crucial in the complicated connection between the initiation of the inflammatory process in the airways and the inhibition of T cell immune response. TGF-β1 is released by fibroblasts, endothelial cells, airway epithelial cells, vascular cells, and

ASM cells.

Nevertheless, inflammatory cells that have migrated, such as eosinophils, are a plentiful source of fibrogenic factors, specifically TGF-β1. TGF-β1 plays a role in the upregulation of extracellular matrix proteins, the development of tissue fibrosis, the production of mucus, and the stimulation of proliferation in ASM cells and fibroblasts[76] .

Matrix Metalloproteinase-9 is related to severe asthma and airway remodeling. Pro-inflammatory cytokine TNF-alpha can regulate MMP-9 production in human bronchial epithelial cells, eosinophils, and myoblasts[77]. TGF-beta 1 stimulates cellular production of MMP-9. The serum levels of MMP-9 of patients with acute severe asthma were also significantly enhanced, compared with stable asthmatic patients[78].

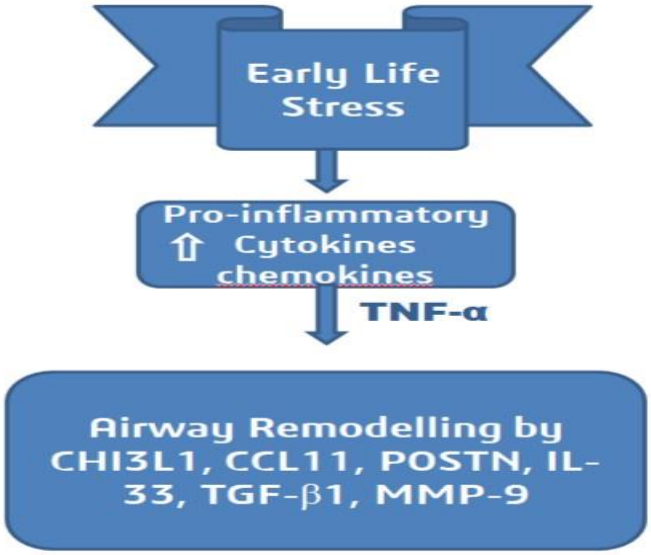

Figure 2.5. Mechanism of early life stress and airway remodeling

Mechanism of early life stress and airway remodeling at a glance in figure 2.5 Early life and chronic stress can affect those genes expression. The hypothesis is early life stress may incresase asthma severity and airway remodeling by elevation *Ccl11*, *Chi3l1*, *Postn* gene expression and in vitro chronic low & high stress can elevate *IL-33*, *CHI3L1, CCL11, POSTN, MMP9 & TGF-B1* gene expression.

3. MATERIAL AND METHODS

3.1 Materials

3.1.1 Equipment

In this scientific experiment, we employed a comprehensive set of specialized equipment, meticulously selected to meet the precise requirements of our study. This included the utilization of the following instruments:

1. Rotor-Gene Q (Qiagen, Germany)
2. Applied Biosystem 7500 fast and 7500 Real time PCR system (Thermo Fisher scientific, USA)
3. T100 Thermal Cycler PCR (Bio-Rad, California, United States)
4. Turbo cycler Thermal cycler (Blue-ray Biotech, Taiwan)
5. Refrigerated Microfuge 20R Centrifuge (Beckman Coulter, United States)
6. Vortex Mixer (SciLogex, United States)
7. Mini Centrifuge (Isolab, Germany)
8. Cold Block (Qiagen, Germany)
9. Micropipettes (Gilson, France)
10. NanoDrop 2000c (Thermo Fisher Scientific, Massachusetts, USA)
11. -20 °C Freezer (Uğur, Türkiye)
12. -80 °C Freezer (Sanyo, Japan)
13. Autoclave (Nüve, Türkiye)
14. Pippettors Finnpipette (Thermo Scientific, USA)
15. Heraeus Multifugu 3SR+ centrifuge (Thermo scientific, USA)
16. Biosafety cabinate Class II Msafe 1200 (Miprolab, Germany)
17. Quantus Flurometer (Promega, USA)

3.1.2. Materials and Reagents

In this scientific experiment, we also employed a diverse selection of specialized materials and reagents, carefully chosen to fulfill the specific requirements of our study. These encompassed a variety of essential components, such as:

1. Eppendorf tubes 1.5 mL (Eppendorf AG, Germany)
2. PCR tubes 0.2 mL
3. Falcon tubes 50 mL
4. 1 mL insulin syringe
5. Micropipette tips with filter (10-100-1000 µL)
6. Chloroform (Merck, Darmstadt, Germany)
7. Ethanol (Merck, Darmstadt, Germany)
8. Isopropanol (Merck, Darmstadt, Germany)
9. Ethylenediamine tetraacetic acid
10. Nuclease-free water (Lonza, Switzerland)
11. QIAzol Lysis Reagent (Qiagen, Germany)
12. RevertAid First Strand cDNA synthesis kit
13. RealQ Plus 2x Master Mix (Ampliqon, Denmark)
14. RPMI 1640 Medium (Cytiva)
15. Fetal Bovine Serum (FBS)
16. Phosphate Buffered Saline 1X PBS (Multicell)
17. Serological pippette (5ml, 10 ml)
18. Dimethylsulfoxide DMSO (Genaxxon, Germany)
19. Dexamethasone (Sigma, Germany)
20. GeneJet RNA purification kit (Thermo Fisher Scientific, USA)
21. BEAS 2B cell line
22. Promega QuantiFluor® RNA System (Promega, USA)

3.2. Methods

The molecular analysis, involving RNA isolation from mice blood, RNA measurement, cDNA synthesis, RT PCR amplification, and agarose gel electrophoresis, was conducted at Ankara Yıldırım Beyazit University's Central Research Laboratory, Application and Research Centre (Merkez Araştırma Laboratuvarı Uygulama ve Araştırma Merkezi-AYBU MERLAB).

Cell culture and incubation with low and high doses of Dexamethasone, RNA isolation from cells, RNA measurement, cDNA synthesis and RT PCR were performed at Saygideger lab, Biotechnology centre, Cukurova University, Adana.

We followed standard laboratory protocols and used validated methods to ensure precise and dependable results.

3.2.1. In Vivo experiments:

The whole procedure is in figure 3.1 at a glance.

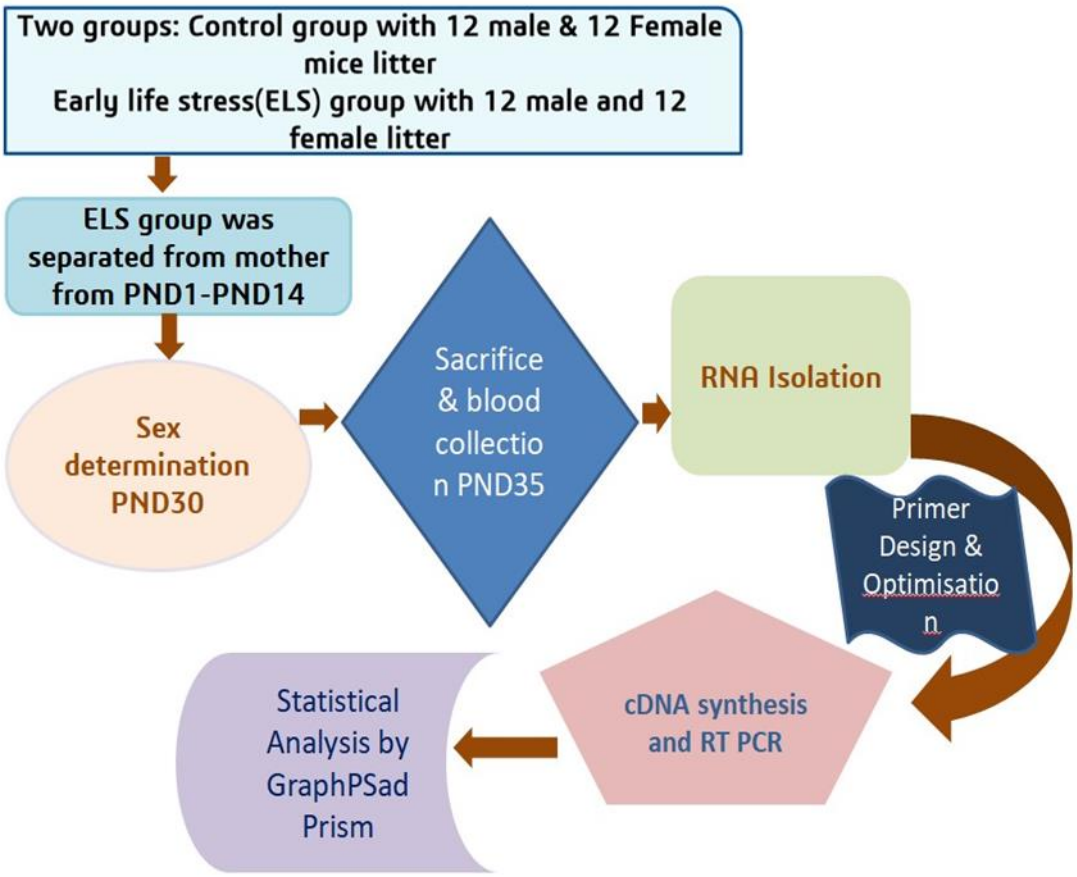

Figure 3.1. In vivo experiments

3.2.1.1. Mice Welfare and Housing Conditions

In this study, *BALB/c* strain mice were utilized as the experimental model. These mice were kept in housing at the Ankara University Faculty of Medicine Laboratory for

Experimental Animals and Research under appropriate conditions. The housing facilities provided for the mice in this study adhered to the requirements of a minimum cage area of 180 cm^2 and a minimum cage height of 12 cm, thus ensuring that their needs were satisfactorily met from birth onwards. The mice were housed in an environment with a temperature range of 20-40 °C and a relative humidity of 45-70%, which is considered optimal for their welfare. (Ethical committee approval number: 2023-16-148).

3.2.1.2. Early life stress by maternal stress procedure

In the study, 8-weeks-old female BALB/c strain mice were divided into two groups: the control group (n=6 females) and for the early life stress (ELS) by unpredictable maternal separation group (n=6 females)

Every female mouse was housed separately in a cage and paired with a male mouse for mating. Following a brief period of mating, the male mice were subsequently extracted from the enclosures, leaving the female mice to endure the entire gestation period in solitude. Weekly cage cleaning was conducted for all groups. The control group encountered negligible disruption, except for cage maintenance. No limitations were placed on the mothers' food and water intake during this time. Despite the presence of maternal stress, the mice offspring persistently consumed milk from their mother.

The early life stress by maternal separation stress procedure involved separating the dams from the litter and placing them in a clean new cage for 3 hours each day of the experiment, at random times from postnatal day 1 (PND1) to PND14.

The determination of the litter was done on postnatal day 30 (PND30). A total of 48 litters were sampled at PND35, consisting of 12 females and 12 males in each group.

3.2.1.3. Sample Collection from Litter

Collection of Blood Samples

1. At PND35, the mice were humanely euthanized through cervical dislocation in accordance with ethical guidelines.
2. The cardiac puncture method was employed to collect blood samples from mice. The experimental procedure involved the utilization of an aseptic technique, whereby a 1 mL insulin syringe (which had been previously cleaned with 0.5 M liquid EDTA) was cautiously introduced into the designated area.
3. A volume of approximately 1 mL of blood was obtained directly from the cardiac region and promptly transferred into a 500 µL QIAzol Lysis Reagent (Qiagen, Germany).
4. The blood sample was subjected to vortexing and subsequently stored at a temperature of -80 °C until the isolation of RNA.

3.2.1.4. RNA Isolation from mice blood

The RNA isolation process was conducted using the phenol-chloroform method with QIAzol lysis reagent (Qiagen, Germany), following the provided protocol.

Sample Homogenization and Phase Separation

* The samples were gradually thawed from -80 ºC to room temperature for RNA Isolation.

* The blood samples underwent vortexing for a duration of 15 seconds. Subsequently, a volume of 100 µL of chloroform was introduced into the solution and subjected to vortexing for a duration of 15 seconds to achieve adequate homogenization.

* The samples were incubated at room temperature for 15 minutes.

* The specimens underwent centrifugation at a force of 12,000 times the acceleration due to gravity for a duration of 15 minutes at a temperature of +4 degrees Celsius. Following the process of centrifugation, a clearly detectable interphase band of a yellowish-whitish shade became visible. The band effectively facilitated the separation of the upper aqueous phase, which encompasses the entirety of the RNA, from the lower organic phase.

* The upper aqueous phase was carefully transferred to a new 1.5 mL Eppendorf

tube, and other phases were carefully avoided during the transfer.

* A volume of 500 µL of isopropanol was added into the aqueous phase. The tube underwent repeated gently up & down every 3 minutes over a 10-minute period while being incubated at a temperature of -20 °C.

* The samples were centrifuged at 12,000 x g for 10 minutes at +4 ºC. After centrifugation, a white pellet, which corresponds to the precipitated RNA, was observed. The size and visibility of the pellet were contingent upon the concentration of RNA.

* The supernatant was subsequently discarded.

* A volume of one milliliter (1 mL) of 75% ethanol was introduced to the pellet. Subsequently, the mixture was briefly vortexed in order to dislodge the pellet.

* The tubes underwent centrifugation at a force of 7,500 times the acceleration due to gravity (7500 × g) for a duration of 5 minutes at a temperature of +4 degrees Celsius.

* The supernatant was discarded.

* The procedure involved the repetition of steps 9 and 10, subsequently followed by the removal of the supernatant. The tubes were allowed to remain uncapped and exposed to ambient temperature for a duration of 5 minutes to facilitate the process of evaporation and achieve dryness.

* The pellet of the blood samples was added by 50 µL of nuclease-free water (NFW) and immediately placed on a refrigerated block.

* The purity and quantity of total RNA were measured using NanoDrop 2000c (Thermo Fisher Scientific, Massachusetts, United States).

3.2.1.5 Nucleic Acid Measurement

The RNA purity and quantity was measured using the NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Massachusetts, United States). The NanoDrop 2000c Spectrophotometer software was used.

* The measuring equipment, such as the micropipette, and the surrounding area of the machine installation underwent sterilization using a solution of 75% ethanol.

* The software on the PC connected to the NanoDrop 2000c Spectrophotometer is utilized for data analysis and processing. The machine was opened.

* The Nucleic Acid application was chosen from the primary menu. Upon the appearance of the wavelength verification window, due diligence was exercised to confirm the arm's position before proceeding to click the OK button.

* The RNA type of the sample to be measured was chosen from the "Type" dropdown menu.

* The concentration units of ng/µL were selected from the drop-down menu adjacent to the concentration box, which was color-coded.

* The experiment utilized a wavelength of 340 nm for bichromate normalization, and the baseline correction box was chosen.

* Prior to the measurement, the checkbox labeled "Add to report" was chosen in order to store the sample data in a workbook.

* The lower pedestal underwent a thorough cleaning process using hydrochloric acid (HCl) and distilled water. 1.5 µL of nuclease-free water was carefully pipetted onto the bottom pedestal, serving as the blanking solution. The arm was carefully lowered, and the designated button was clicked, initiating the measurement process. The blank solution is commonly associated with the buffer in which the molecule of interest is suspended or dissolved.

The RNA concentration of the blood samples was found to be between indicating that they are suitable for cDNA synthesis.

* The measurement report has been successfully saved to the specified folder. After the measurement, the bottom pedestal was carefully cleaned using lint-free tissue.

* The initial sample was assigned a unique identification number. Before transferring the sample into the bottom pedestal, a gentle tap was applied to the bottom of the sample tube to ensure adequate mixing. The sample was carefully pipetted onto the bottom pedestal, with a volume of 1.5 µL. Subsequently, the arm was lowered to initiate the measurement process. The aforementioned procedure was conducted for each of the samples. A dry laboratory wipe was used to clean the upper and lower pedestals after each sample. A measurement was conducted at regular intervals of 30 minutes throughout the

entire measurement process. Following the completion of the measurements, the pedestal was carefully cleansed using distilled water.

* The concentration of RNA in the blood samples was determined to be above 1 pg and below 1 µg, suggesting their suitability for cDNA synthesis.

* The 260/280 ratio is commonly observed to be 2.0 or greater, whereas the 260/230 ratio typically falls within the range of 1.8-2.2. The 260/280 ratio is a comparison of the absorbance at 260 nm, which is associated with nucleic acid absorption, and 280 nm, which is related to protein absorption. An approximate ratio of 2.0 signifies the presence of pure RNA, while a lower ratio implies the existence of protein or other impurities.

The 260/230 ratio allows for the comparison of absorbance at 260 nm (which is associated with nucleic acid absorption) and 230 nm (which is related to contaminant absorption). This ratio provides valuable insights into the purity of RNA samples. Higher 260/230 ratios are indicative of RNA samples that are more pristine.
The RNA concentration of the blood samples was found to be between indicating that they are suitable for cDNA synthesis.

3.2.1.6. cDNA Synthesis

The cDNA was synthesized using Thermo Scientific RevertAid First Strand cDNA Synthesis kit according to the manufacturer's protocol. A total of 500ng of total RNA was used. The reaction was carried out in a thermocycler (Bio-Rad, California, United States) with the program provided in **Table 3.1** All reactions were set up on a cold block to minimize the risk of RNA degradation. After adding all the mixture, they were spinned well to avoid bubbles before incubating in thermocycler. Thermocycler program is in **Table 3.2**.

Table 3.1. cDNA synthesis

Name	Amount
Total RNA	2 µL (500ng)
Oligo $(dT)_{18}$ primer	1 µL
Random Hexamer primer	1 µL
Nuclease-free water	8 µL
5X Reaction Buffer	4 µL
Ribolock RNase Inhibitor (20 U/µL)	1 µL
10 mM dNTP Mix	2 µL
RevertAid RT (200 U/µL)	1 µL
Total volume	**20 µL**

Table 3.2. Thermocycler program for cDNA Synthesis: (T100 Thermal Cycler PCR)

Temperature ˚C	Time
25	5 min
42	60 min
70	5 min
4	∞

.

3.2.1.7. RT PCR for mRNA Quantification

RT PCR was performed using Rotor-Gene Q (Qiagen, Germany) and the RealQ Plus 2X mastermix Green (Ampliqon, Denmark). The master mix was prepared according to the specifications provided in **Table 3.3.,** then it was distributed to each tube and placed into Rotor-Gene Q (Qiagen, Germany) and run using program described in **Table 3.4.** The gene-specific primer pairs listed in **Table 3.5.** were used for amplification. The primer pairs used in the qRT PCR experiments were carefully designed using primer designing software and multiple molecular databases.

Table 3.3. Mix composition for RT PCR

Component	Volume/Reaction
RealQ Plus 2X Master Mix Green	**12.5 µL**
Nucleas-free water	**9.5 µL**
Forward and Reverse Primer Mix (5 µM)	**1 µL**
cDNA	**2 µL**
Total volume	**25 µL**

Table 3.4. Thermal cycling profile in Rotor gene (Qiagen, Germany):

Parameter	**Value**
Run	Two steps with melt
Sample volume	25 µL

Step	**Hold**	**Cycling**					**Melt**
		45 cycles					
		Denaturation	Annealing				
			Postn	**Chi3l1**	**Ccl11**	**Gapdh**	
Time	15 min	20 sec	60 sec	60 sec	60 sec	60 sec	90s (1st) 5s (2nd)
Temp ˚C	95	95	58	58	58	60	Ramp from 65-95˚C

Table 3.5. Sequences of forward and reverse primers (Mice) used for RT-PCR reaction**.**

Gene symbol	Forward Primer	Reverse Primer	Product length (base pair)
Gapdh	CTCTCTGCTCCTCCCTGTTC	TACGGCCAAATCCGTTCACA	105
Postn	GACCTGCAATGACGAAGATCC	TTTAATGACTGGCTCTCCGTG	104
Chi3l1	TGAAGACCAGAAACACCAACC	CAAAGCCATAAGAACGCAGGA	150
Ccl11	AACTTCCTGCTGCTTTATCATGAC	CCTGGTCTTGAAGACTATGGC	115

3.2.2. In vitro

The whole procedure is in figure 3.2 at a glance.

Beas 2B cell line was used to see the effect of dexamethasone. The Beas 2B cell line was gifted by Hasan Bayram Hoca from Koc University.

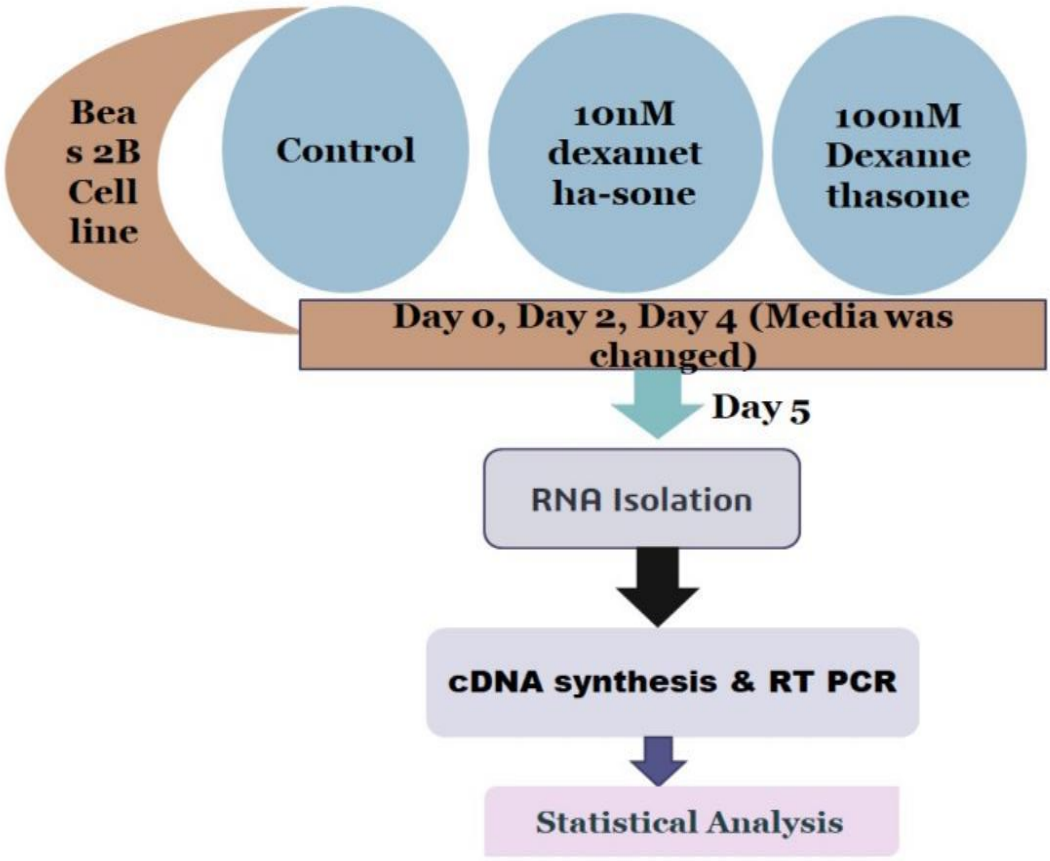

Figure 3.2. In vitro experiments

3.2.2.1. Cell culture

Beas 2B cell line was cultured in RPMI 1640 media (with 10% FBS) in a 75 cm Flask at 37° C in a humidified 95% air–5% CO2 incubator. In 3rd day, when the confluency was 90%, the old media was discarded. After washing with PBS, 0.6 ml Trypsin was added and incubated in 37ºC. After 5 minutes when the cells were floated and detached from base, 2.4 ml media was added in the flaks. Cells with media were divided into three 6 cm culture dishes. One is for control group: one is for low concentration of dexamethasone and one is for high concentration of dexamethasone.

3.2.2.2 Dexamethasone administration:

When the concentration of cell in each culture dish were near 80-85%, the day is considered as day 0.

The media was discarded and washed with PBS for two times.

In control group: 4995 uL RPMI 1640(with 10% FBS) and 5uL DMSO were added.
In low concentrated dexamethasone group: 4995uL RPMI 1640 (with 10% FBS) and 5ul 10nM dexamethasone were added.
In high concentrated dexamethasone group: 4995uL RPMI 1640 (with 10% FBS) and 5ul 100nM dexamethasone were added.
The procedures were repeated on day 2 and day 4 also. On day 5, all cells were collected for RNA isolation.

3.2.2.3. RNA isolation by GeneJet RNA purification kit

* The cells were resuspended by 600 µL lysis buffer in the 2ml effendorf.
* After 10 seconds vortexing for 3 times, 360µL Absolute Ethanol were added.
*After pippetting very well, 700µL from this mixture were taken in the filtered collection tube. It was centrifuged for 1 minute in 14000RPM at 25ºC.
* After discarding the supernatant, the left 260µL from the mixture were taken in the same collection tube and were centrifuged.
* After discarding supernant, 700µL wash buffer-1 was added and centrifuged at 14000 RPM for 1 minute.
* After discarding supernatant, 600µL Wash Buffer-2 was added and centrifuged at 1400RPM for 1 minute.
* The supernatant was discarded and 250µL Wash buffer-2 was added again and centrifuged for 2 min at 14000RPM.
* The collection tubes were discarded and new 1.5 ml effendorfs were taken. The filters were placed in the effendorfs.
* 50µL nuclease free water was added and centrifuged at 14000RPM for 1 minute, The filters were discarded. And the effendorfs with pallet were immediately placed in the cold block and then placed in -70ºC.

3.2.2.4. RNA quantification by Quantifluor RNA System

*** 1x TE:** 20x TE buffer was diluted with nuclease free water to 1x TE and taken in a 2ml effendorf tube.

* **Prepare working solution for high standard calibration:** The Quantifluor RNA dye was diluted in 1x TE buffer. In 2000µL 1x TE, 5µL dye was added.

* **Prepare blank:** 200µL of Quantifluor RNA dye working solution was taken in a empty 0.5ml PCR tube and the tube was protected from light.

* **Prepare standard:** A 500ng standard was prepared by adding 5µL of the provided RNA standard to 200µL of Quantifluor RNA dye working solution in an empty 0.5ml PCR tube. It was mixed well and protected from light.

* **Prepare sample:** RNA 1µL sample was added to 200µL of QuatiFluor RNA Dye working solution in a 0.5ml PCR tubes. The tube was vortexed well and protected from light.

* The prepared samples were incubated at room temperature for 5 minutes at dark.

* In Quantus Flurometer, RNA protocol was selected. Blank and standard samples were calibrated and saved.

* Then the unknown samples were measured and recorded the sample concentrations for further use.

3.2.2.5. cDNA synthesis

cDNA was synthesized in Turbo Cycler PCR by revertaid cDNA synthesis kit same as

3.2.2.6. RT PCR

RT PCR were conducted in Applied biosystem 7500 fast and 7500 RT PCR system. the realQ Plus 2X mastermix Green (Ampliqon, Denmark). The master mix was prepared according to the specifications provided in **Table 3.6.,** then it was distributed to each wells and placed into Applied biosystem and run using program described in **Table 3.7.** The gene-specific primer pairs listed in **Table 3.8.** were used for amplification. The

primer pairs used in the qRT PCR experiments were carefully designed using primer designing software and multiple molecular databases.

Table 3.6. Mix composition for RT PCR

Component	Volume/Reaction
RealQ Plus 2X Master Mix Green	**8 µL**
Nucleas-free water	**10 µL**
Forward Primer (10 µM)	**0.5 µL**
Reverse Primer (10 µM)	**0.5 µL**
cDNA	**1 µL**
Total volume	**20 µL**

Table 3.7. Thermal cycling profile in Applied biosystem 7500 and 7500 fast Real Time PCR

Parameter	Value
Run	Two steps with melt
Sample volume	20 µL

Step	**Hold**	**Cycling**					**Melt**
Hold 1: 50ºC, 02.00min		40 cycles					
Hold 2: 95ºC, 10.00min		Denaturation	Annealing				
				CHI3L1	**CCL11**	**18S**	
				TGFB1	**MMP9**	**POSTN**	
Time	50ºC, 02.00 min	Time	15sec	60 sec	60 sec	60 sec	15 sec
Tempº C	95ºC, 10.00 min	TempºC	95	60	60	60	95

Table 3.8. Sequences of forward and reverse primers (Human) used for RT-PCR reaction.

Gene symbol	Forward Primer (5'..3')	Reverse Primer (3'.5')	Product length (base pair)
18S	CTTAGAGGGACAAGTGGCG	ACGCTGAGCCAGTCAGTGTA	107
POSTN	GGATCAGCGCCTCCTTAAAT	CCTCCGATGGTTTCCAGTATT	102
CHI3L1	TGTCGGAGGATGGAACTTTG	ATGGCGGTACTGACTTGATG	92
CCL11	GGTGCAGGATTCCATGAAGTAT	TCAGGCTCTGGTTTGGTTTC	90
MMP9	TCACTTTCCTGGGTAAGGAGTA	CTGTCAAAGTTCGAGGTGGTAG	91
IL33	GTGACGGTGTTGATGGTAAGA	CTCCACAGAGTGTTCCTTGTT	92
TGFB1	TTCAGTCACCATAGCAACACTC	GAACTCCTCCCTTAACCTCTCT	96

3.3. Data analysis

The delta-delta Ct method, ($\mathbf{2^{-\Delta\Delta Ct}}$) was used in order to calculate the relative fold gene expression of samples[79].

At first, the triplicate ct values for mice blood & BEAS 2B samples were averaged. The following formula was utilized then:

(1) ΔCt = Ct (gene of interest) – Ct (housekeeping gene)

(2) Average of Control samples of gene of interest

(3) ΔΔCt = ΔCt (treated sample) – ΔCt of average of control samples.

(4) Finally, in order to determine the fold gene expression, we performed the calculation of 2 to the power of negative ΔΔCt,

So, Fold gene expression = $2^{-(\Delta\Delta Ct)}$

The data analysis and graphical representation in this study were performed using GraphPad Prism 8.4.3 software. For fold change analysis, control value is one. The gene expression compared to control value from o.5 to 1.5 is considered as no alteration. So, more than 1.5 fold change is considered as significantly overexpression and less than 0.5 fold change is considered as significantly down regulated. The data were presented as mean ±SD.

4. RESULTS

In vivo study involved female BALB/c mice that were divided into two groups: the control group (n=6) and the MS (unpredictable maternal separation) group (n=6). Mice were then mated with male mice to initiate pregnancy. After the mice became pregnant and gave birth, the stress procedures were initiated. The ELS group was directly exposed to the MS group dams and their corresponding litter. The mRNA expression study, only the litter (n=48) were included, with an equal representation of 12 male and 12 female litter per group.

In vitro study was involved with BEAS 2B cell line in three groups, control, Low concentrated (10 nM) dexamethasone group and high concentrated (100nM) dexamethasone group.

4.1. mRNA Expression in mice blood Analysis Results

The fold differences in mRNA expression levels of *Ccl1*, *Postn* and *Chi3l1* between the early life stress (ELS) and the control group were analysed. Comparisons were made between the female ELS group and the female control group, as well as between the male ELS group and the male control group.

4.1.1. ELS causes *Ccl11* overexpression in female

The findings revealed a 4.32 fold change in *Ccl11* mRNA expression between the female ELS group and their respective control group. However, no significant difference was observed in the male ELS group compared to their control group (1.02 fold). These results are in **Figure 4.1**.

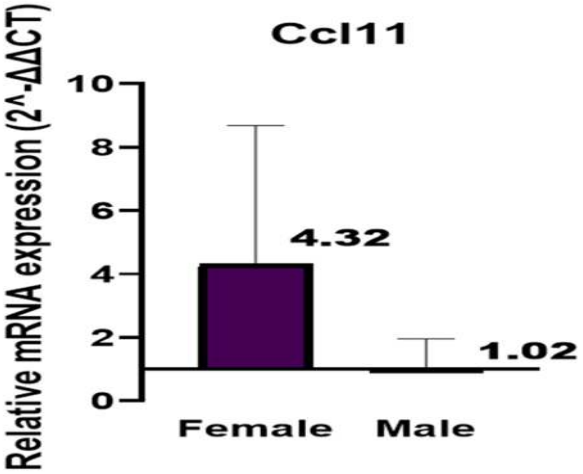

Figure 4.1. Bar graph showing fold change of mRNA expression levels of *Ccl11* in females and males by comparing with their respective control groups.

4.1.2 ELS causes *Postn* overexpression:

The findings revealed a 18.69 & 28.62 fold difference respectively in *Postn* mRNA expression between the female & male ELS group and their respective control group. These results are in **Figure 4.2.**

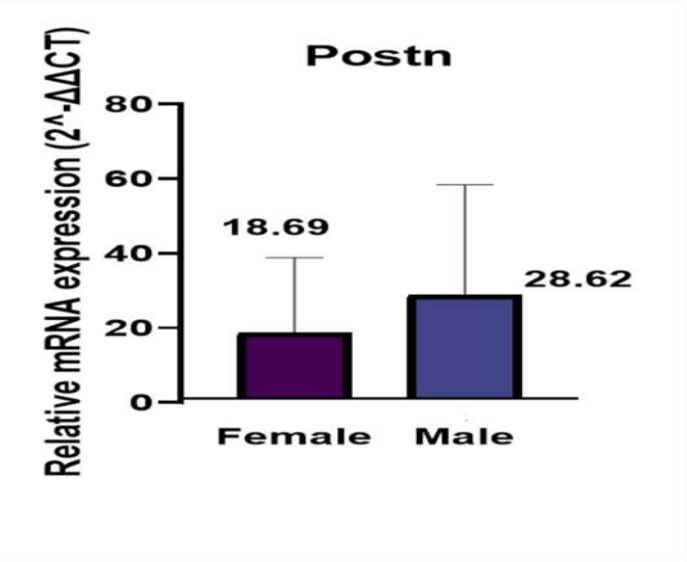

Figure 4.2. Bar graph showing fold change of mRNA expression levels of *Postn* in females and males by comparing with their respective control groups.

4.1.3 ELS causes *Chi3l1* 0verexpression:

The findings revealed 2.30 fold difference in *Chi3l1* mRNA expression between the female ELS group and their respective control group and mild upregulation (1.65 fold change) in the male ELS group compared to their control group. These results are in **Figure 4.3**

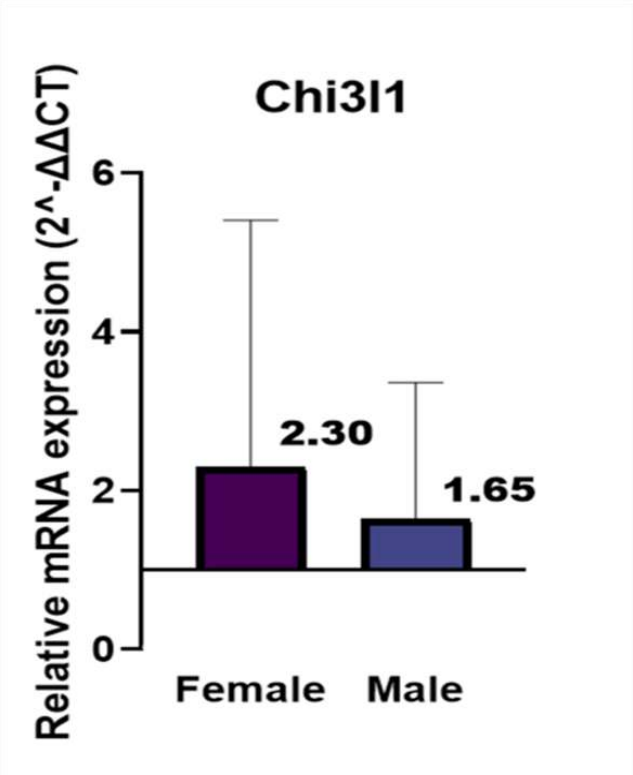

Figure 4.3. Bar graph showing fold change of mRNA expression levels of *Chi3l1* in females and males by comparing with their respective control groups.

4.2. In vitro study mRNA expression analysis results

4.2.1. Low & high stress mimic on BEAS 2B cause overexpressed *IL33*:

The findings revealed that chronic low dose(10nM) dexamethasone significantly increased the expression of *IL33* mRNA, with a fold change of 4.9 and chronic high dose(100nM) dexamethasone significantly increased with a fold change of 3.28. The result is in **Figure 4.4**.

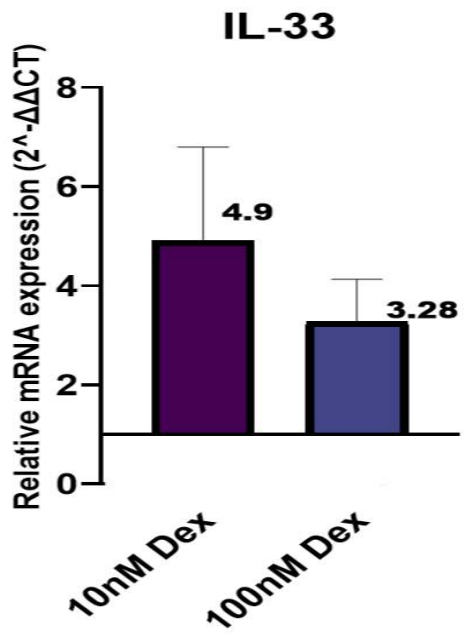

Figure 4.4. The results show significant overexpression of *IL-33*.

4.2.2. Effect of low & high stress mimic on BEAS 2B for *CCL11*& *CHI3L1* cause no alteration

Chronic low stress (10nM dexamethasone)& chonic high stress (100nM Dexamethasone) causes no altered expression (respectively 0.80 & 0.95 fold expression) of *CCL11* compared to control and *CHI3L1* showed a fold change of 1.08 and 0.98 respectively of mRNA expression. The result is in **Figure 4.5**.

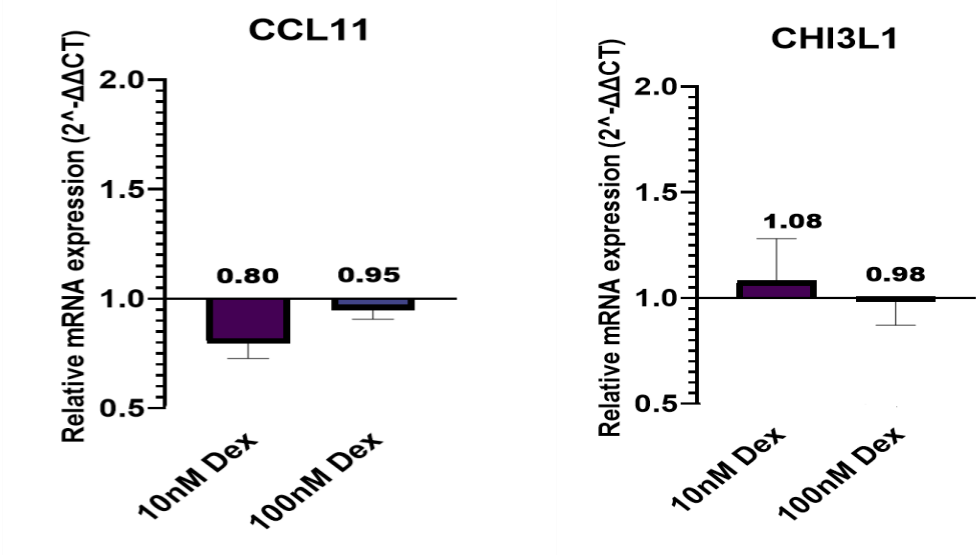

Figure 4.5. The results show no significant alteration of *Ccl11 & CHI3L1*.

4.2.3. Effect of low & high stress mimic on BEAS 2B for POSTN and MMP9 cause down regulation

The findings revealed that chronic low dose(10nM) dexamethasone causes down regulation of the expression of *POSTN* & *MMP9* mRNA, with a fold change of 0.26 & 0.12 and chronic high dose(100nM) dexamethasone with a fold change of 0.23 & 0.06 respectively. The result is in **Figure 4.6**.

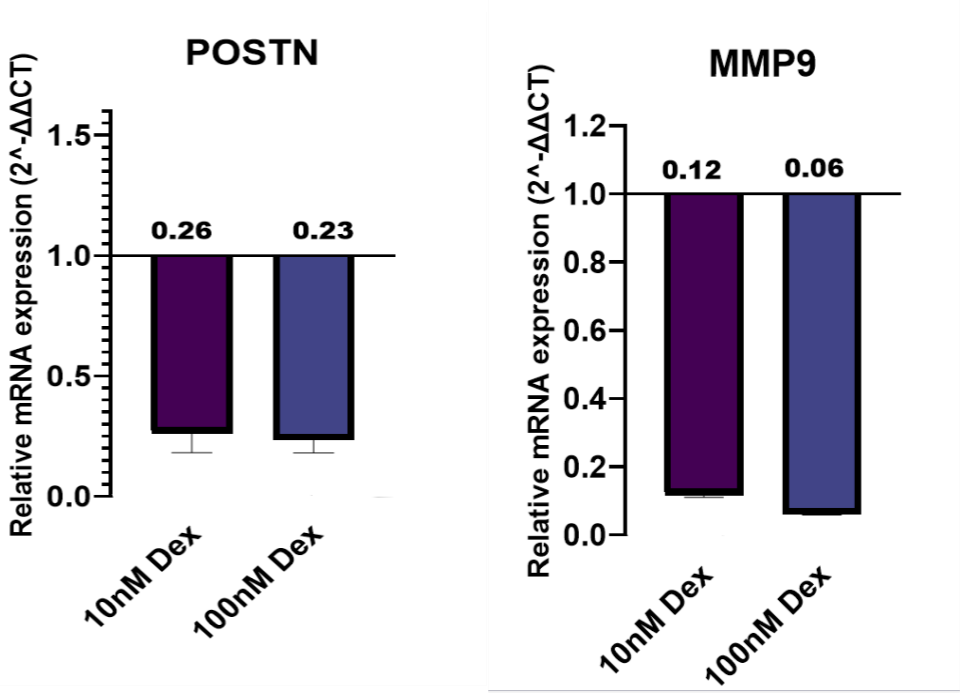

Figure 4.6. The results showed down regulation of *POSTN* & *MMP9*.

4.2.4 Effect of low stress mimic causes no alteration but high stress mimic causes *TGF-β1* mild down regulation on Beas 2B cell

The results showed chronic low (10nM) Dexamethasone causes no alteration and chronic high (100nM) dexamethasone cause mild down regulation (0.81&0.35 fold). The result is in **Figure 4.7**.

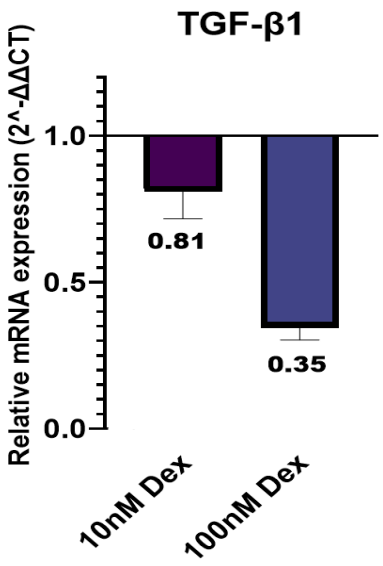

Figure 4.7. The results showed chronic low stress cause no alteration (0.81 fold) but chronic high dexamethasone cause mild down regulation 0.35 fold.

5. DISCUSSION

This study utilized an in-vivo early life stress mice model, utilizing the method of maternal separation. Additionally, an in-vitro stress model was established by conducting low and high doses of dexamethasone to a cell culture of Beas 2Bs. The findings suggest that there is a significant change between in vivo female control group and ELS group *Ccl11* expression and both male & female group of *Postn* & *Chi3l1*. *IL-33* exhibited a significant overexpression of 4.9 and 3.28 fold in both low stress and high stress conditions, mimicking cortisol levels. POSTN and MMP9 showed a significant down regulation of 0.26 fold and 0.12 fold in case of low stress mimic and 0.23 & 0.06 fold in case of high stress mimic condition respectively.

The most employed pre-clinical method to study the connection between early life chronic stress and its effects on mental and physical health is through repeated maternal separation[80]. The application of the Maternal Separation paradigm in rodents has become a highly appropriate method for simulating Early Life Stress (ELS) in humans. The neonatal offspring are subjected to a daily separation from their mothers lasting 3 hours, beginning on the second day after birth and continuing until the fourteenth day[81,82,83]. Maternal separation as early life stress shows effect on airway inflammation[84]. To establish the experimental model, Human bronchial epithelial cell line Beas 2B was used and dexamethasone was utilized. When an individual experiences a prolonged condition of low stress and a prolonged state of high stress, the cortisol levels are equivalent to 10nM and 100nM dexamethasone, respectively[85]. So, here two different doses, 10nM and 100nM of dexamethasone were administered in the culture of Beas 2B cell.

The results indicate a notable difference in the expression of *Ccl11* between the female control group and the female ELS group in vivo.. But there is no significant change in the expression of male control and male ELS group. But no statistically significant differences were found in the expression of *Postn* in female or male ELS mice compared to the control group in our study. Previous study has shown that, in females, the HPA axis is triggered more quickly and generates a greater amount of stress hormones compared to males[38].

Periostin is one of the most highly expressed genes in asthma[67]. It serves as an indicator for diseases mediated by Th2 cytokines. Both IL-13 and IL-4 can stimulate the release of periostin from lung fibroblasts, while IL-13 specifically triggers the production of periostin by epithelial cells. Periostin additionally triggers TGF-β signaling, which can further stimulate the deposition of ECM and the remodeling of the airways[86]. Serum periostin is particularly elevated in the eosinophilic/type 2 subtype of severe asthma[20].
The most potent chemokine for eosinophil migration, CCL11 (eotaxin-1), is released by bronchial epithelial and endothelial cells, and eosinophils have high expression of CCR3, the chemokine's receptor[87]. It is also confirmed that CCL11 stimulates eosinophils by causing RNase and cell-free granules to be released[88]. When compared to healthy people, asthmatic patients' blood, sputum, and exhaled breath condensate (EBC) all showed a significant rise in CCL11 levels[21].

In low stress and high stress mimicing cortisol level, IL-33 has overexpressed significantly 4.9 and 3.28 fold. IL-33 played a pivotal role in the inflammation of the airways. Multiple studies have demonstrated a significant correlation between the IL-33/ST2 pathway and the severity of asthma[89]. IL-33 is generated in the early stages of allergic inflammation and stimulates Th2 activation via type 2 innate lymphoid cells, which express IL-13 and IL-4 and enhance type-2 immune response[90]. Prior studies have shown that the level of IL-33 in the serum is increased in individuals with asthma[91]. The bronchial epithelium serves as a significant storage site for IL-33 in the lung[92]. So, our result is significantly related to airway remodeling and asthma severity.

We have some limitations in our study, the mice were asthmatic or not, it was not diagnosed. But on the other hand, another study has shown that mice cannot develop spontaneous asthma; it is needed to initiate asthma in mice[93]. The in vitro experiments were performed on only Beas 2B cell line, but experiments should be performed on other cell lines like airway smooth muscle cell, fibroblast and other immune cells. The protein levels were not measured. For in vitro chronic low & high stress mimic condition, dexamethasone may administered for more than 5 days.

6. CONCLUSION

Airway remodeling and severe asthma related genes, *Ccl11*, *Chi3l1* & *Postn* are increased in early life chronic stressed mice and *IL-33* is elevated in chronic low and high stress mimic condition. Our findings are correlate with airway remodeling and early life chronic stress.

7. REFERENCES

1. **Haktanir Abul M, Phipatanakul W**. Severe asthma in children: Evaluation and management. *Allergol Int*. **2019**;68(2):150-157.

2. **Chung KF, Wenzel SE, Brozek JL**, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J*.

3. S**ettipane RA, Kreindler JL, Chung Y, Tkacz J**. Evaluating direct costs and productivity losses of patients with asthma receiving GINA 4/5 therapy in the United States. *Ann allergy, asthma Immunol Off Publ Am Coll Allergy, Asthma, Immunol*. **2019**;123(6):564-572.e3.

4. Y**aribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A**. The impact of stress on body function: A review. *EXCLI J*. **2017**;16:1057-1072.

5. **Schneiderman N, Ironson G, Siegel SD**. Stress and Health: Psychological, Behavioral, and Biological Determinants. *Annu Rev Clin Psychol*. **2005**;1(1):607-628.

6. **Nater UM, Skoluda N, Strahler J.** Biomarkers of stress in behavioural medicine. *Curr Opin Psychiatry*. **2013**;26(5):440-445.

7. **Kudielka BM, Wüst S**. Human models in acute and chronic stress: assessing determinants of individual hypothalamus-pituitary-adrenal axis activity and reactivity. *Stress*. **2010**;13(1):1-14.

8. **Smith SM, Vale WW**. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci*. **2006**;8(4):383-395.

9. **Morey JN, Boggero IA, Scott AB, Segerstrom SC**. Current Directions in Stress and Human Immune Function. *Curr Opin Psychol*. **2015**;5:13-17.

10. **Won E, Kim YK**. Stress, the Autonomic Nervous System, and the Immune-kynurenine Pathway in the Etiology of Depression. *Curr Neuropharmacol*. **2016**;14(7):665-673.

11. **Oswald LM, Zandi P, Nestadt G, Potash JB, Kalaydjian AE, Wand GS**. Relationship between cortisol responses to stress and personality. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol*. **2006**;31(7):1583-1591.

12. **Olff M**. Stress, depression and immunity: the role of defense and coping styles. *Psychiatry Res*. **1999**;85(1):7-15.

13. **Noushad S, Ahmed S, Ansari B, Mustafa UH, Saleem Y, Hazrat H**. Physiological biomarkers of chronic stress: A systematic review. *Int J Health Sci (Qassim)*. **2021**;15(5):46-59.

14. **Buchmann AF, Kopf D, Westphal S**, et al. Impact of early parental child-rearing behavior on young adults' cardiometabolic risk profile: a prospective study. *Psychosom Med*. **2010**;72(2):156-162.

15. **Palumbo ML, Prochnik A, Wald MR, Genaro AM**. Chronic Stress and Glucocorticoid Receptor Resistance in Asthma. *Clin Ther*. **2020**;42(6):993-1006.

16. **Lumertz F, Kestering É, Orso R,** et al. Effects of Early Life Stress on Brain Cytokines: A Systematic Review and Meta-Analysis of Rodent Studies. *Neurosci Biobehav Rev*. **2022**;139:104746.

17. **Baumeister D, Akhtar R, Ciufolini S, Pariante CM, Mondelli V**. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. *Mol Psychiatry*. **2016**;21(5):642-649.

18. **Ji L, Liu Y, Liu P**, et al. Serum periostin and TNF-α levels in patients with obstructive sleep apnea-hypopnea syndrome. *Sleep Breath*. **2021**;25(1):331-337.

19. **Post S, Rozeveld D, Jonker MR, Bischoff R, Van Oosterhout AJ, Heijink IH**. ADAM10 mediates the house dust mite-induced release of chemokine ligand CCL20 by airway epithelium. *Allergy Eur J Allergy Clin Immunol*. **2015**;70(12):1545-1552.

20. **Matsumoto H**. Role of serum periostin in the management of asthma and its comorbidities. *Respir Investig*. **2020**;58(3):144-154.

21. **Wu D, Zhou J, Bi H**, et al. CCL11 as a potential diagnostic marker for asthma? *J asthma Off J Assoc Care Asthma*. **2014**;51(8):847-854.

22. **Jin Y, Song J, Xu F**, et al. Association between YKL-40 and asthma: a systematic meta-analysis. *Sleep Breath*. **2022**;26(3):1011-1022.

23. **Zhao R, Shi Y, Liu N, Li B.** Elevated levels of interleukin-33 are associated with asthma: A meta-analysis. *Immunity, Inflamm Dis*. **2023;**11(4):e842.

24. **Al-Alawi M, Hassan T, Chotirmall SH**. Transforming growth factor β and severe asthma: A perfect storm. *Respir Med*. **2014**;108(10):1409-1423.

25. **Pascoe, C. D., Obeidat, M., Arsenault, B. A., Nie, Y., Warner, S., Stefanowicz, D., Wadsworth, S. J., Hirota, J. A., Jasemine Yang, S., Dorscheid, D. R., Carlsten, C., Hackett, T. L., Seow, C. Y., & Paré, P. D.** Gene expression analysis in asthma using a targeted multiplex array. *BMC Pulmonary Medicine. **2017**;17*(1), 189.

26. **Yang, T., Li, Y., Lyu, Z., Huang, K., Corrigan, C. J., Ying, S., Wang, W., & Wang, C**.Characteristics of proinflammatory cytokines and chemokines in airways of asthmatics: Relationships with disease severity and infiltration of inflammatory cells. *Chinese Medical Journal. **2017**; 130*(17), 2033–2040.

27. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet (London, England)*. **2017**;390(10100):1151-1210.

28. **Bousquet, J., Mantzouranis, E., Cruz, A. A., Aït-Khaled, N., Baena-Cagnani, C. E., Bleecker, E. R., Brightling, C. E., Burney, P., Bush, A., Busse, W. W., Casale, T. B., Chan-Yeung, M., Chen, R., Chowdhury, B., Chung, K. F., Dahl, R., Drazen, J. M., Fabbri, L. M., Holgate, S. T., … Zuberbier, T.** Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma. In *The Journal of allergy and clinical immunology. **2010**;* (Vol. 126, Issue 5, pp. 926–938).

29. **Hekking, P.-P. W., Wener, R. R., Amelink, M., Zwinderman, A. H., Bouvy, M. L., & Bel, E. H.** The prevalence of severe refractory asthma. *The Journal of Allergy and Clinical Immunology. **2015**; 135*(4), 896–902.

30. **Busse, W. W., Banks-Schlegel, S., & Wenzel, S. E**. Pathophysiology of severe asthma. *Journal of Allergy and Clinical Immunology. **2000** 106*(6), 1033–1042.

31. **Calhoun, W. J., & Chupp, G. L.** The new era of add-on asthma treatments: where do we stand? *Allergy, Asthma and Clinical Immunology. **2022**; 18*(1), 1–13.

32. **Scotney, E., & Saglani, S**. Diagnosis and Management of Problematic Severe Asthma. *Acta Medica Academica. **2020**; 49*(2), 117–129.

33. **Varricchi, G., Ferri, S., Pepys, J., Poto, R., Spadaro, G., Nappi, E., Paoletti, G., Virchow, J. C., Heffler, E., & Canonica, W. G.** Biologics and airway remodeling in severe asthma. *Allergy: European Journal of Allergy and Clinical Immunology.* ***2022****; 77*(12), 3538–3552.

34. **Pu, Q., Zhao, Y., Sun, Y., Huang, T., Lin, P., Zhou, C., Qin, S., Singh, B. B., & Wu, M**. TRPC1 intensifies house dust mite-induced airway remodeling by facilitating epithelial-to-mesenchymal transition and STAT3/NF-κB signaling. *FASEB Journal.* ***2019****; 33*(1), 1074–1085.

35. **Lloyd, C. M., Robinson, D. S., & McMillan, S. J**. Modelling airway remodelling in asthma. *Drug Discovery Today: Disease Models.* ***2004****; 1*(4), 425–430.

36. **Salter, B., Pray, C., Radford, K., Martin, J., & Nair, P**. Regulation of human airway smooth muscle cell migration and relevance to asthma. **2017;** *Respiratory Research*, *18*, 156.

37. **Saglani, S., & Lloyd, C. M.** Novel concepts in airway inflammation and remodelling in asthma. *European Respiratory Journal.* ***2015****; 46*(6), 1796–1804.

38. **Leistner, C., & Menke, A**. Hypothalamic–pituitary–adrenal axis and stress. In *Handbook of Clinical Neurology.* ***2020****;* (1st ed., Vol. 175). Elsevier B.V.

39. **Liu, Y. Z., Wang, Y. X., & Jiang, C. L**. Inflammation: The common pathway of stress-related diseases. *Frontiers in Human Neuroscience.* ***2017****; 11*(June), 1–11.

40. **Johnson, S.** In Times of Adversity: A Neuroscience Perspective on Stress, Health, and Implications for Society Post-pandemic. *Yale Journal of Biology and Medicine.* ***2022****; 95*(1), 165–170.

41. **Petrescu, A. D., Kain, J., Liere, V., Heavener, T., & DeMorrow, S**. Hypothalamus-Pituitary-Adrenal Dysfunction in Cholestatic Liver Disease. *Frontiers in Endocrinology.* ***2018****; 9*(November), 1–15.

42. **Barnthouse, M., & Jones, B. L**. The Impact of Environmental Chronic and Toxic Stress on Asthma. *Clinical Reviews in Allergy and Immunology.* ***2019****; 57*(3), 427–438.

43. **Miller, G. E., & Chen, E**. Life stress and diminished expression of genes encoding glucocorticoid receptor and beta2-adrenergic receptor in children with asthma. *Proceedings of the National Academy of Sciences of the United States of America.* ***2006****; 103*(14), 5496–5501.

44. **Graham, Y. P., Heim, C., Goodman, S. H., Miller, A. H., & Nemeroff, C. B.** The effects of neonatal stress on brain development: implications for psychopathology. *Development and Psychopathology.* ***1999****; 11*(3), 545–565

45. **Bolt, R. J., van Weissenbruch, M. M., Lafeber, H. N., & Delemarre-van de Waal, H**. A. Glucocorticoids and lung development in the fetus and preterm infant. *Pediatric Pulmonology.* ***2001****; 32*(1), 76–91.

46. **Mehls, O., Himmele, R., Hömme, M., Kiepe, D., & Klaus, G.** The interaction of glucocorticoids with the growth hormone-insulin-like growth factor axis and its effects on growth plate chondrocytes and bone cells. *Journal of Pediatric Endocrinology & Metabolism.* ***2001****; JPEM*, *14 Suppl 6*, 1475–1482.

47. **Yates, D. A., Gareau, L. G., So, J. D., Johan, D., Yates, D. A., Yang, P., Macqueen, G., & Perdue, M. H**. Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress. *American Journal of Physiology - Gastrointestinal and Liver Physiology.* ***2002****; 283*, 1257–1263.

48. **Romeo, R. D., Mueller, A., Sisti, H. M., Ogawa, S., McEwen, B. S., & Brake, W. G.** Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. *Hormones and Behavior.* ***2003****; 43*(5), 561–567.

49. **Parfitt, D. B., Levin, J. K., Saltstein, K. P., Klayman, A. S., Greer, L. M., & Helmreich, D. L.** Differential early rearing environments can accentuate or attenuate the responses to stress in male C57BL/6 mice. *Brain Research.* ***2004****; 1016*(1), 111–118.

50. **Romeo, R. D., Fossella, J. A., Bateup, H. S., Sisti, H. M., Brake, W. G., & McEwen, B. S.** Maternal separation suppresses TGF alpha mRNA expression in the prefrontal cortex of male and female neonatal C57BL/6 mice. *Brain Research. Developmental Brain Research.* ***2004;*** *152*(1), 73–77

51. **Barreau, F., Ferrier, L., Fioramonti, J., & Bueno, L.** Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier and mucosal immunity in rats. *Gut.* ***2004;*** *53*(4), 501–506.

52. **Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G**. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience.* ***2010****; 170*(4), 1179–1188.

53. **O'Mahony, S. M., Hyland, N. P., Dinan, T. G., & Cryan, J. F**. Maternal separation as a model of brain-gut axis dysfunction. *Psychopharmacology.* ***2011****; 214*(1), 71–88.

54. **Chida, Y., Sudo, N., Sonoda, J., Hiramoto, T., & Kubo, C**. Early-life psychological stress exacerbates adult mouse asthma via the hypothalamus-pituitary-adrenal axis. *American Journal of Respiratory and Critical Care Medicine.* ***2017****; 175*(4), 316–322.

55. **Yonas, M. A., Lange, N. E., & Celedón, J. C.** Psychosocial stress and asthma morbidity. *Current Opinion in Allergy and Clinical Immunology.* ***2012;*** *12*(2), 202–210.

56. **Alvarez, P., Green, P. G., & Levine, J. D.** Stress in the adult rat exacerbates muscle pain induced by early-life stress. *Biological Psychiatry.* ***2013*** *74*(9), 688–695.

57. **Dzubur, E., & Li, M**. *and Asthma Symptoms in Hispanic Adolescents*. **2017** *40*, 1–20.

58. **Bellin, M., Osteen, P., Collins, K., Butz, A., Land, C., & Kub, J.** The influence of community violence and protective factors on asthma morbidity and healthcare utilization in high-risk children. *Journal of Urban Health : Bulletin of the New York Academy of Medicine.* ***2014****; 91*(4), 677–689.

59. **Koinis-Mitchell, D., Kopel, S. J., Salcedo, L., McCue, C., & McQuaid, E. L.** Asthma indicators and neighborhood and family stressors related to urban living in children. *American Journal of Health Behavior.* ***2014****; 38*(1), 22–30.

60. **Koinis-Mitchell, D., McQuaid, E. L., Seifer, R., Kopel, S. J., Esteban, C., Canino, G., Garcia-Coll, C., Klein, R., & Fritz, G. K.** Multiple urban and asthma-related risks and their association with asthma morbidity in children. *Journal of Pediatric Psychology.* ***2007****; 32*(5), 582–595.

61. **Smejda, K., Polanska, K., Merecz-Kot, D., Krol, A., Hanke, W., Jerzynska, J., Stelmach, W., Majak, P., & Stelmach, I**. Maternal Stress During Pregnancy and Allergic Diseases in Children During the First Year of Life. *Respiratory Care.* ***2018****; 63*(1), 70–76.

62. **Hartwig, I. R. V., Sly, P. D., Schmidt, L. A., Van Lieshout, R. J., Bienenstock, J., Holt, P. G., & Arck, P. C.** Prenatal adverse life events increase the risk for atopic diseases in children, which is enhanced in the absence of a maternal atopic predisposition. *Journal of Allergy and Clinical Immunology.* ***2014****; 134*(1), 160-169.e7.

63. **Suglia, S. F., Duarte, C. S., Sandel, M. T., & Wright, R. J.** Social and environmental stressors in the home and childhood asthma. *Journal of Epidemiology and Community Health.* ***2010****; 64*(7), 636–642.

64. **Saygideger, Y., Özkan, H., Baydar, O., & Yilmaz, O.** Adulthood asthma as a consequence of childhood adversity: a systematic review of epigenetically affected genes. *Journal of Developmental Origins of Health and Disease.* ***2022;*** *13*(6), 674–682.

65. **Fehrenbach, H., Wagner, C., & Wegmann, M**. Airway remodeling in asthma: what really matters. *Cell and Tissue Research.* ***2017*** *367*(3), 551–569.

66. **Li, W., Gao, P., Zhi, Y., Xu, W., Wu, Y., Yin, J., & Zhang, J**. Periostin: Its role in asthma and its potential as a diagnostic or therapeutic target. *Respiratory Research.* ***2015****; 16*(1), 1–10. https://doi.org/10.1186/s12931-015-0218-2

67. **Woodruff, P. G., Boushey, H. A., Dolganov, G. M., Barker, C. S., Yee, H. Y., Donnelly, S., Ellwanger, A., Sidhu, S. S., Dao-Pick, T. P., Pantoja, C., Erle, D. J., Yamamoto, K. R., & Fahy, J. V.** Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proceedings of the National Academy of Sciences of the United States of America.* ***2007****; 104*(40), 15858–15863.

68. **Puxeddu, I., Bader, R., Piliponsky, A. M., Reich, R., Levi-Schaffer, F., & Berkman, N**. The CC chemokine eotaxin/CCL11 has a selective profibrogenic effect on human lung fibroblasts. *Journal of Allergy and Clinical Immunology.* ***2006****; 117*(1), 103–110.

69. **Saunders, R., Sutcliffe, A., Woodman, L., Kaur, D., Siddiqui, S., & Okayama, Y.** *Europe PMC Funders Group The airway smooth muscle CCR3 / CCL11 axis is inhibited by mast cells*. **2014**; *63*(9), 1148–1155.

70. **Gerthoffer, W. T.** Migration of airway smooth muscle cells. *Proceedings of the American Thoracic Society.* ***2008****; 5*(1), 97–105.

71. **Park, J.-A., Drazen, J. M., & Tschumperlin, D. J.** The chitinase-like protein YKL-40 is secreted by airway epithelial cells at base line and in response to compressive mechanical stress. *The Journal of Biological Chemistry.* ***2010****; 285*(39), 29817–29825.

72. **Bara, I., Ozier, A., Girodet, P. O., Carvalho, G., Cattiaux, J., Begueret, H., Thumerel, M., Ousova, O., Kolbeck, R., Coyle, A. J., Woods, J., Tunon De Lara, J. M., Marthan, R., & Berger, P.** Role of YKL-40 in bronchial smooth muscle remodeling in asthma. *American Journal of Respiratory and Critical Care Medicine.* ***2012****; 185*(7), 715–722.

73. **Konradsen, J. R., James, A., Nordlund, B., Reinius, L. E., Söderhäll, C., Melén, E., Wheelock, A. M., Lödrup Carlsen, K. C., Lidegran, M., Verhoek, M., Boot, R. G., Dahlén, B., Dahlén, S. E., & Hedlin, G.** The chitinase-like protein YKL-40: a possible biomarker of inflammation and airway remodeling in severe pediatric asthma. *The Journal of Allergy and Clinical Immunology.* ***2013****; 132*(2), 328-35.e5.

74. **Chupp, G. L., Lee, C. G., Jarjour, N., Shim, Y. M., Holm, C. T., He, S., Dziura, J. D., Reed, J., Coyle, A. J., Kiener, P., Cullen, M., Grandsaigne, M., Dombret, M.-C., Aubier, M., Pretolani, M., & Elias, J. A.** A chitinase-like protein in the lung and circulation of patients with severe asthma. *The New England Journal of Medicine.* ***2007****; 357*(20), 2016–2027.

75. **Sjöberg, L. C., Nilsson, A. Z., Lei, Y., Gregory, J. A., Adner, M., & Nilsson, G. P**. Interleukin 33 exacerbates antigen driven airway hyperresponsiveness, inflammation and remodeling in a mouse model of asthma. *Scientific Reports.* ***2017****; 7*(1), 1–10.

76. **Janulaityte, I., Januskevicius, A., Kalinauskaite-Zukauske, V., Bajoriuniene, I., & Malakauskas, K.** In vivo allergen-activated eosinophils promote collagen I and fibronectin gene expression in airway smooth muscle cells via TGF-β1 signaling pathway in Asthma. *International Journal of Molecular Sciences.* ***2020****; 21*(5), 1–19

77. **Ohbayashi, H., & Shimokata, K**. Matrix metalloproteinase-9 and airway remodeling in asthma. *Current Drug Targets: Inflammation and Allergy.* ***2005****; 4*(2), 177–181. https://doi.org/10.2174/1568010053586246.

78. **Kim, H.-S., Shang, T., Chen, Z., Pflugfelder, S. C., & Li, D.-Q**. TGF-beta1 stimulates production of gelatinase (MMP-9), collagenases (MMP-1, -13) and stromelysins (MMP-3, -10, -11) by human corneal epithelial cells. **2004**; *Experimental Eye Research*, *79*(2), 263–274

79. **Livak, K. J., & Schmittgen, T. D.** Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods.* ***2001****; 25*(4), 402–408.

80. **Dutcher, E. G., Pama, E. A. C., Lynall, M.-E., Khan, S., Clatworthy, M. R., Robbins, T. W., Bullmore, E. T., & Dalley, J. W.** Early-life stress and inflammation: A systematic review of a key experimental approach in rodents. *Brain and Neuroscience Advances.* ***2020****; 4*, 239821282097804

81. **Loria, A. S., Brands, M. W., Pollock, D. M., & Pollock, J. S**. Early life stress sensitizes the renal and systemic sympathetic system in rats. *American Journal of Physiology. Renal Physiology.* ***2013****; 305*(3), F390-5

82. **Lehmann, J., Stöhr, T., & Feldon, J.** Long-term effects of prenatal stress experience and postnatal maternal separation on emotionality and attentional processes. *Behavioural Brain Research.* ***2000****; 107*(1–2), 133–144.

83. **Lippmann, M., Bress, A., Nemeroff, C. B., Plotsky, P. M., & Monteggia, L. M.** Long-term behavioural and molecular alterations associated with maternal separation in rats. *European Journal of Neuroscience.* ***2007****; 25*(10), 3091–3098.

84. **Ouchi, R., Kawano, T., Yoshida, H., Ishii, M., Miyasaka, T., Ohkawara, Y., Takayanagi, M., Takahashi, T., & Ohno, I**. Maternal separation as early-life stress causes enhanced allergic airway responses by inhibiting respiratory tolerance in mice. *Tohoku Journal of Experimental Medicine.* ***2018****; 246*(3), 155–165.

85. **Xiang, L., & Marshall, G. D.** Immunomodulatory effects of dexamethasone on gene expression of cytokine and stress hormone receptors in peripheral blood mononuclear cells. *International Immunopharmacology.* ***2013****; 17*(3), 556–560.

86. **O'Dwyer, D. N., & Moore, B. B.** The role of periostin in lung fibrosis and airway remodeling. *Cellular and Molecular Life Sciences : CMLS.* ***2017****; 74*(23), 4305–4314.

87. **Smit, J. J., & Lukacs, N. W.** A closer look at chemokines and their role in asthmatic responses. *European Journal of Pharmacology.* ***2006****; 533*(1), 277–288.

88. **Shamri, R., Melo, R. C. N., Young, K. M., Bivas-Benita, M., Xenakis, J. J., Spencer, L. A., & Weller, P. F**. CCL11 elicits secretion of RNases from mouse eosinophils and their cell-free granules. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology.* ***2012****; 26*(5), 2084–2093.

89. **Oshikawa, K., Kuroiwa, K., Tago, K., Iwahana, H., Yanagisawa, K., Ohno, S., Tominaga, S. I., & Sugiyama, Y**. Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. *American Journal of Respiratory and Critical Care Medicine.* ***2001****; 164*(2), 277–281

90. **Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., Zurawski, G., Moshrefi, M., Qin, J., Li, X., Gorman, D. M., Bazan, J. F., & Kastelein, R. A.** IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity.* ***2005****; 23*(5), 479–490.

91. **Gon, Y., & Hashimoto, S.** Role of airway epithelial barrier dysfunction in pathogenesis of asthma. *Allergology International.* ***2018****; 67*(1), 12–17

92. **Préfontaine, D., Nadigel, J., Chouiali, F., Audusseau, S., Semlali, A., Chakir, J., Martin, J. G., & Hamid, Q.** Increased IL-33 expression by epithelial cells in bronchial asthma. In *The Journal of allergy and clinical immunology.* ***2010****;* (Vol. 125, Issue 3, pp. 752–754).

93. **Aun, M. V., Bonamichi-Santos, R., Arantes-Costa, F. M., Kalil, J., & Giavina-Bianchi, P.** Animal models of asthma: utility and limitations. *Journal of Asthma and Allergy.* ***2017****; 10*, 293–301.

APPENDICES

Appendix-1. Ethical statement for animal model studies

T.C.
ANKARA ÜNİVERSİTESİ REKTÖRLÜĞÜ
Hayvan Deneyleri Yerel Etik Kurulu

HAYVAN DENEYLERİ YEREL ETİK KURULU KARARI

TOPLANTI TARİHİ : 13/09/2023
TOPLANTI NO : 2023-16
DOSYA NO : 2023-114
KARAR NO : 2023-16-148

Yürütücülüğünü Ankara Yıldırım Beyazıt Üniversitesi Tıp Fakültesi Tıbbi Genetik Anabilim Dalı öğretim üyelerinden Dr. Öğr. Üyesi Keziban Korkmaz BAYRAM'ın yaptığı, araştırıcı olarak Sehreen TORY, Md. Mojahidur HASAN, Aida Nurul BAROKAH, Uzm. Dr. Arslan BAYRAM ve Doç. Dr. Yasemin SAYGIDEĞER'in katıldığı "The impact of early stress on airway remodeling and severe asthma related gene expression profile in mice" başlıklı araştırma projesinin içeriği Üniversite Senatosunun 12/2/2016 tarihli toplantısında 430/3642 sayılı kararı ile kabul edilen ve Hayvan Deneyleri Merkezi Etik Kurulu'nun 19/2/2016 tarih ve 42 sayılı kararı ile onaylanan "Ankara Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu Yönergesi"ne göre değerlendirilmiş ve söz konusu projenin aşağıda belirtilen kapsamda düzeltildikten sonra yeniden değerlendirilmesine oy birliği ile karar verilmiştir.

1- Araştırmanın başlığı Türkçe yazılmalıdır.
2- Proje yürütücüsünün ikinci danışman olduğunu gösterir resmi yazının bir örneğinin başvuru formuna eklenmesi gerekmektedir.
3- Başvuru formu tam ve eksiksiz olarak doldurulmalı, canlı hayvan kullanılacağı anlamı çıkan ifadeler düzeltilmelidir (Örn. Başvuru formu 7, A51, E1, E2 gibi).

ETİK KURUL ÜYELERİ				
Unvanı / Adı / Soyadı	Uzmanlık Dalı	Kurumu	Cinsiyeti	İmza
Prof. Dr. M. Taner KARAOĞLU (Başkan)	Viroloji Anabilim Dalı	Veteriner Fakültesi	E	
Prof. Dr. Tanju ÖZÇELİKAY (Başkan Vekili)	Farmakoloji Anabilim Dalı	Eczacılık Fakültesi	E	
Prof. Dr. Emine DEMİREL YILMAZ (Üye)	Tıbbi Farmakoloji Anabilim Dalı	Tıp Fakültesi	K	
Prof. Dr. Nuri YİĞİT (Üye)	Zooloji Anabilim Dalı	Fen Fakültesi	E	

Adres: Ankara Üniversitesi Rektörlüğü 06100 - Tandoğan / ANKARA **Tel:** 0 (312) 212 60 40 / 2183

Appendix-2. Thesis similarity report

CUKUROVA UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
MS/PHD/
THESIS STUDY SMILARITY REPORT FORM
☐**BEFORE DEFENSE** ☐√ **AFTER DEFENSE**

CUKUROVA UNIVERSITY
TO THE DIRECTORATE OF THE INSTITUTE OF MEDICAL SCIENCES

Date:13/02/2024

Thesis Title / Subject:
The impact of early life stress on airway remodeling and severe asthma related gene expression profile in mice

Plagiarism Program Outcome Evaluation RESULT: 6%

Similarity ratio:
(Explanation is required if the similarity rate is above the accepted limits.)

I examined the Cukurova University, the Institute of Medical Sciences Application Principles for Obtaining and Using the Thesis Study Affinity Report, and according to the maximum similarity rates specified in these Application Principles, my thesis does not contain any plagiarism; I declare that I accept all kinds of legal responsibility that may arise in a possible situation where the opposite is determined and that the information I have given above is correct.
I respectfully submit it.

Sehreen Tory
13/02/2024

Name: Sehreen Tory
Student number: 2021921018
Major / Major Art Branch: Translational Medicine
Degree: Master's

EXPLANATION (*If the similarity rate is **above the accepted limits**, it is mandatory to make the necessary explanation to this part.*)

CURRICULUM VITAE

Sehreen Tory, Completed her high school in 2011 with GPA 5.00 out of GPA 5.00. She earned her MBBS (Bachelor of Medicine & Bachelor of Surgery) degree from Rajshahi University in 2017. She is a registered medical doctor in Bangladesh. She graduated from Cukurova Ubiversity, Adana, Turkiye in 2024 with an MSc (Translational Medicine) with a cGPA 3.77 out of cGPA 4.00. She has gained many laboratory techniques such as cell culture, MTT assay, DNA isolation, RNA isolation, Protein isolation, PCR, Peripheral blood mononuclear cells isolation, SDS Page, Gel electrophoresis etc. She loves pets.