Republic of Iraq
Ministry of Higher Education and
Scientific Research
University of Kerbala
College of Pharmacy
Department of Pharmacology and
Toxicology

Effects of Genetic Polymorphisms of OATP2B1 Transporter and CYP2C8 Metabolizing Enzyme on Montelukast Therapy Response in Asthmatic Children in Kerbela Province

A Thesis

Submitted to the Council of College of Pharmacy / University of Kerbala as a Partial Fulfillment of the Requirements for the Master Degree in Pharmacology and Toxicology

By

Hasnaa Haider Mohammed

B.Sc. in Pharmacy (University of Kerbala, 2016)

Supervised by

Professor Dr.
Uday Abdul-Reda Hussein

Professor Dr.
Hassan Mahmood Mousa

2024 A.D. **1445 A.H**

صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

سورة طه من الآية ١١٤

Supervisor certification

We certify that this thesis entitled **(Effects of Genetic Polymorphisms of OATP2B1 Transporter and CYP2C8 Metabolizing Enzyme on Montelukast Therapy Response in Asthmatic Children)** was prepared by **(Hasnaa Haider Mohammed)** under our supervision at the University of Kerbala / College of Pharmacy, as a partial fulfillment of the requirement for the degree of Master in Pharmacology and Toxicology.

Supervisor

Professor Dr.

Uday Abdul-Reda Hussein

Ph.D. Pharmacology and Therapeutics

University of Al-Ameed

Supervisor

Professor Dr.

Hassan Mahmoud Mousa

Ph.D. Genetic Engineering and Biotechnology

University of Kerbala

In the view of the available recommendation, I forward this M.Sc. thesis for debate by the examining committee.

Assistant Professor Dr.

Amal Umran Mousa

M.Sc. Toxicology

Head of the Department of Pharmacology and Toxicology

University of Kerbala / College of Pharmacy

Committee Certification

We, the examining committee, certify that we have read this thesis entitled (**Effects of Genetic Polymorphisms of OATP2B1 Transporter and CYP2C8 Metabolizing Enzyme on Montelukast Therapy Response in Asthmatic Children**), and have examined the student (**Hasnaa Haider Mohammed**) in its contents, found it adequate and with standing as a thesis for the Degree of Master of Pharmacology and Toxicology.

Chairman
Professor Dr.
Ahmed Salih Sahib

Member
Assistant Professor Dr.
Amal Umran Mousa

Member
Ass. Professor Dr.
Fatima Adnan Ali

Supervisor
Professor Dr.
Uday Abdul-Reda Hussein

Supervisor
Professor Dr.
Hassan Mahmoud Mousa

Approved by

College of Pharmacy/ University of Kerbala
As a thesis for degree of
Master of Science in Pharmacology and Toxicology

Asst Prof. Dr.
Mohammed Ibrahim Rasool
Dean
College of Pharmacy / University of Kerbala

Seal
Higher studies registration
College of Pharmacy / University of Kerbala

Dedication

To the people who are suffering from any disease,

To my dear parents, who supported and guided me to the useful knowledge when I was child,

To all the people in my life and touch my heart.

Hasnaa

Acknowledgements

First of all, I render my thankfulness to ALLAH who gave me ability, self-confidence, and strength to finish this study. Without his care this would have never been happened in reality.

I owe a deep gratefulness to the College of Pharmacy at University of Kerbala, Department of Pharmacology and Toxicology for giving me a chance to complete this study.

I would like to express my deep sense of gratitude to the Dean of College of Pharmacy Assistant Professor Dr. Mohammed Ibrahim Rassoul and my first supervisor Prof. Dr. Uday Abdul-Reda Hussein for his continuous support, vast knowledge and patience.

I am deeply grateful to my co-supervisor Prof. Dr. Hassan Mahmood Mousa Abo Al-Maali for his endless support, and for sharing his profound knowledge.

My appreciation is to chairman of the Pharmacology and Toxicology Department in the College of Pharmacy, Asst. Prof. Amal Umran for her nice support and encouragement. Thanks is extended for all the staff of Department of Pharmacology and Toxicology for their generous support.

My deep appreciation goes to Prof. Dr. Ahmed Salih and Prof. Dr. Mazin Hamid Ouda in the college of pharmacy for their assistant, support, and inducement.

I render my gratitude to Dr. Akeel Mahdi, Dr. Haider Faegh and Dr. Maryam Zuhair and all medical and laboratory staff at Kerbala respiratory center for their help during the clinical study.

Great thanks go to all lovely children participated in this study and their respected parents, with my wishes for all to get healthy and happy life.

List of Contents

List of Tables		
Table number	**Title**	**Page**
1.1.	Recommended dose of Montelukast	23
2.1.	Chemicals and kits	34
2.2.	Instruments	35
2.3.	Primer sequence of OATP2B1 transporter gene c.935G>A polymorphism	44
2.4.	Primer sequence of CYP2C8*1B enzyme gene g.-271C>A polymorphism	44
2.5.	Lists of requirements for OATPO2B1transporter gene c.935G>A optimization	45
2.6.	Condition for CYP2C8*1B enzyme gene g.-271C>A optimization	46
3.1.	Demographics characteristics of the asthmatic children	48
3.2.	Asthma control test in asthmatic children	51
3.3.	Pulmonary Function Test (PFT) among controlled and uncontrolled groups of asthmatic children	51
3.4.	Serum Total Immunoglobulin E levels among controlled and uncontrolled groups of asthmatic children	52
3.5.	Distribution of different genotype of OATP2B1 transporter of asthmatic children	53
3.6	Hardy-Weinberg Equilibrium for OATP2B1 c.935G>A transporter gene polymorphism of asthmatic children	55

List of Figures		
Figure number	**Title**	**Page**
1.1.	Volume-Time curve from a Forced Expiratory maneuver	13
1.2.	The paths of cysteinyl leukotrienes	18
1.3.	The chemical structure of montelukast	19
1.4.	Predicated 2-D structure of OAT2B1 full length transcription variant	27
1.5.	Oxidative mechanism of Montelukast	28
3.1.	Electrophoresis of agarose gel for allele specific PCR product of OATP2B1 transporter gene c.935G>A polymorphisms	54
3.2.	Electrophoresis of agarose gel for allele specific PCR product of CYP2C8*1B enzyme gene g.-271C>A polymorphism	56

List of Abbreviations	
Abbreviation	**Meaning**
AA	Arachidonic acid
ACT	Asthma Control Test
BMI	Body Mass Index
Bp	Base pair
cAMP	cyclic Adenosine Mono Phosphate
Cys LTR	Cysteinyl Leukotrienes Receptor
EDTA	Ethylene diamine tetra Acetic acid
EIB	Exercise Induce Bronchospasm
ELISA	Enzyme-linked immunosorbent assay
FEV	Force Expiratory Volume
FEV1	Force Expiratory Volume in One second
FRC	Functional Residual Capacity
FRV	Functional Residual Volume
5-LO	5-Lipooxygenase
FLAP	5-Lipooxygenase Activating Protein
GINA	Global Initiative for Asthma
GM-CSF	granulocyte-macrophage colony-stimulating factor
ICAM-1	Intercellular Adhesion Molecule-1
ICS	Inhaled Corticosteroid
Ig E	Immunoglobulin E
IL	Interleukin
LABA	Long Acting β2 Agonist
LT	Leukotrienes
LTRA	Leukotriene Receptor
M3	Muscarinic receptor
MDI	Metered Dose Inhaler
MLLT3	mixed-lineage leukemia gene or myeloid/lymphoid translocated to, 3, super elongation complex subunit
NF-B	Nuclear Factor kappa light chain enhancer of activated B cells
NHLBI	National Heart, Lung, and Blood Institute's Guidelines

OAT2B1	Organic-anion-transporting polypeptide
OCS	Oral Corticosteroid
PCR	Polymerase Chain Reaction
PEF	Peak Expiration Flow
PFT	Pulmonary function test
Rs	Reference SNP cluster ID to identify specific SNP
RV	Residual volume
SABA	Short Acting β2 Agonist
SNP	Single nucleotide polymorphisms
SLCO2B1	Solute Carrier Organic Anion Transporter Family Member 2B1)
TBE	Tries Borate EDTA
Th2	T-helper type 2 lymphocytes
TLC	Total lung capacity
VC	Volume capacity

Abstract:

Background: Montelukast, a cysteinyl leukotriene 1 receptor antagonist, approved for both acute and chronic therapy of asthma in adults and children.

A nonsynonymous single nucleotide polymorphism (SNP) in the organic anion transporting polypeptide 2B1 (OATP2B1), (rs12422149) c.935G>A, and CYP2C8*1B (rs7909236) g.-271 enzyme have been associated with reduced plasma concentrations of montelukast in patients with asthma.

Aims of the study:

The study aims to investigate the effects of genetic polymorphisms of OATP2B1 c.935G>A (rs12422149) transporter and CYP2C8*1B (rs7909236) metabolizing enzyme polymorphism on montelukast therapy response in asthmatic children.

Patients and methods: This cross-sectional observational study is done on Respiratory clinic center of Karbala hospital of children, from end of October 2022 to the end of September 2023. One hundred male and female, aged between 6 to 15 years old and taken montelukast as maintenance therapy daily for at least one month, were enrolled in the study. Pulmonary Function test (PFT), Asthma Control Test (ACT), and total serum Immunoglobulin E were measured. Allele specific polymerase chain reaction (AS-PCR) had been done to detect different patients' genotypes after DNA extraction.

Results: The results of this study show that the distribution of OATP2B1 transporter gene c.935G>A polymorphism was 48(48%) for wild homozygous GG, 45(45%) for mutant heterozygous GA, and 7 (7%) for mutant homozygous AA. This polymorphism was estimated to have a significant association with montelukast therapy response and the patients who have wild homozygous GG genotype, show better response to montelukast therapy that indicated by improvement in pulmonary function test, reduction of serum total Ig E level, and improvement of asthma

symptom in asthma control test (score ≥20) when compared with mutant genotypes (GA and AA).

Regarding to CYP2C8*1B g.-271C>A gene polymorphism, the result of current study shows no significant association of CYP2C8*1B polymorphism with montelukast therapy response. The distribution of CYP2C8*1B g.-271C>A gene was as follows; 74(74%) for CC homozygous wild type, 22 (22%) for CA heterogeneous mutant and 4 (4%) for AA homozygous mutant types.

Conclusions: The polymorphism of OATP2B1 transporter affects the response of asthmatic children to montelukast therapy and asthmatic children having wild GG genotype showed better improvement in lung function and clinical condition (reduction of serum total IgE level and ≥20 score of asthma control test) after treatment in comparison with those having mutant AA and GA genotypes in mild to moderate asthma.

The CYP2C8*1B g.-271C>A genetic polymorphism was not significantly associated with montelukast therapy responses despite its existence in Iraqi asthmatic patients.

Chapter One

Introduction

1. Introduction

1.1. Definition of asthma

The Global Initiative for Asthma (GINA), defined asthma as “a multiple disorder, that mainly associated with persistent airway inflammation with the history of respiratory symptoms such as chest tightness, shortness of breath, wheeze, and cough that change in intensity in over time, as well as accompanied by varying expiratory airflow limitations"(GINA, 2023; Lupu *et al*., 2023). Asthma manifested in all ages, but it is typically first demonstrating in childhood. Adult and pediatric asthma are very different from one another. The majority of differences seen in the disease’s prognosis, diagnosis, therapy, and epidemiology (Kliegman *et al*., 2020). Childhood asthma is still a heterogeneous disorder. It is crucial to conduct additional research regarding the immunopathology and genetic roots of pediatrics abnormalities in order to personalized therapy (Reddy *et al*.,2016).

1.2. Asthma phenotypes

Asthma phenotypes are identifiable groups of demographics, clinical, and/or pathophysiology sign. There are several clinical phenotypes, among the most typical are (GINA, 2023):

• **Allergic asthma:** The most readily identifiable type of asthma is this type. It often first demonstrates in infancy and is associated to a previous or past family history of allergic conditions including allergic rhinitis, eczema, and drug or food allergies. Before getting therapy, check-up of the developed mucus from these persons frequently shows eosinophilic airway inflammation. This phenotype of asthmatic patients frequently reacts good to inhaled corticosteroids (ICS)therapy (Bel *et al.*, 2004). In children, (allergic) asthma is the highest prevalent chronic respiratory condition and is studied extensively (Schiffers et al.,2023).

• **Early onset asthma:** A small percentage of people suffer from asthma that is unrelated to allergies. The patient's sputum of these groups may have an eosinophilic or neutrophilic cells or just have a slight number of inflammatory cells (paucigranulocytic). In this group patients typically slowly respond to ICS short-term therapy (Moore et al., 2010).

• **Delayed onset asthma:** In some people, mostly women, experience delayed-onset asthma for the first time. The individuals of this groups frequently have non-allergic tendencies, need higher ICS dosages, or respond inefficiently to corticosteroid therapy. When a patient has delayed-onset asthma, work-related asthma, or asthma triggered by pollutants at work, should be ruled out (Wenzel et al., 2012; Ricciardolo *et al.,*2023).

• **Asthma that cause persistent airflow limitation:** Nearly individuals with chronic asthma experience persistent or only partially reversible airflow limitation. This is considered to be produced by remodelling of airway wall (Wookfork *et al.,*2023).

• **Obesity and asthma:** Obesity has been implicated as a risk factor for allergic diseases, based on the potential for adiposity to promote a pro-inflammatory state (Chang et al.,2023). Adipose tissue is important in regulation of inflammation and obesity could affect the inflammation homeostasis. Changes in cytokine levels as well as altered immune responses have been suggested as potential mechanisms relating obesity to asthma. Adipocytes and adipose tissue macrophages produce pro-inflammatory cytokines such as IL-6 which has been found in increased levels in asthmatics and has been related to low lung function (Björkander *et al.,*2023). Some obese asthmatic patients exhibit severe respiratory symptoms but less eosinophilic airway inflammation (GINA,2023).

1.3. Asthma epidemiology

Worldwide, paediatric asthma is a serious public health concern. Asthma now affects approximately 300 million people worldwide, and based on rising trends, that quantity is predicted to rise to 400 million in 2025. Asthma causes up to 250,000 deaths annually, the majority of which are preventable. Internationally, 0 to 0.7 deaths per 100,000 individuals are attributed to childhood asthma. Asthma is one of the most common allergic and chronic diseases of childhood and among the top 20 illnesses in the world for years of life lost to disability in children and considered the most prevalent chronic illness in children (Serebrisky *et al.*, 2019; Altaş *et al.*, 2023). The prevalence differs among various nations, affecting 2-22% of the population (Strömberg, 2023). The disease is more prevalent in developed countries with the highest rate seen in Australia (21.5%), Sweden (20.2%), United Kingdom (18.2%), Canada (14.1%), and the United States of America (10.9%) (Iqbal, *et al*.,2014; Lundbäck, *et al*.,2016).

According to study in children of Yemen (Faisal, *et al*.,2009) with ages 6-7 and ages 13-14 the asthma prevalence was (9.4% and 8.8%) respectively. In Syrian children, the prevalence of 6-7 years was (4.7% - 5.7%) and (3.9% -6.5%) for 13- 14 years old (Mohammad, *et al.,*2010). A further study, conducted in Egypt in 2016 with children ages 6 to 12 found a prevalence rate of 6.3%. In Saudi Arabia, limited epidemiological studies have been carried out to determine the frequency of childhood asthma, and the outcomes of those research have been remarkably variable (Alahmadi *et al*., 2019). In Rabigh that considered to have the second highest prevalence of asthma in Saudi children, after Hofuf in the eastern region of Saudi Arabia, with a prevalence of 33.7%, recorded by a standardized questionnaire (Alahmadi et al., 2023).

The prevalence and incidence of asthma in Baghdad; in one research involving 3360 primary school students, the prevalence of asthma was

16.4%. (Al-Thamiri *et al.,* 2005). According to Al Samari *et al.* (2019), the overall incidence of asthma was estimated to be 7.2 when determined by direct questioning but it was 8.9% when determined by checking each year over 200,000 individuals with asthma in Iraq are either hospitalized or treated in a hospital emergency room.

1.4. Asthma etiology

The origin of asthma is complicated and still not entirely understood. Multiple genetic and environmental factors are known to interact in a complicated manner. There is evidence that there is a genetic factor in asthma development, and the most recent research indicates that many genes may contribute to the onset of asthma. Asthma risk may be influenced by a variety of environmental factors, many of which have been discovered. These include dietary factors, exposure to allergies, infectious diseases, chemicals used at work, and air pollution (Whittlesea *et al.,*2019).

The main risk factor for the development of asthma is atopy. Atopy is defined as genetic tendency for the development of an immunoglobulin E (Ig E)-mediated response to distinctive aeroallergens, that is frequently linked to childhood-onset asthma. Although atopy and adult-onset asthma may be related, many persons with asthma have no family history of the condition and have tested negative for common aeroallergens on skin tests. Nasal polyps, aspirin sensitivity, and sinusitis are possible conditions among some of these people. Major risk factors for the asthma onset include atopy and exposure to occupational chemical sensitizers, but there are also a number of other factors that may make people more susceptible to developing the condition. Viral infections, tiny stature at birth, nutrition, exposure to tobacco smoking, and environmental toxins are a few of these causes (Zeind *et al*. ,2018).

The “hygiene theory “, which suggests that decreasing exposure to infectious organisms in early childhood increases vulnerability to allergic illnesses, is one explanation for the rising prevalence of asthma. Air pollution is one of the additional explanations that could account for the rise in asthma cases in emerging nations (Whittlesea *et al*.,2019).

1.5. Asthma pathophysiology

Asthma is associated with inflammation and airway constriction, mainly in the medium sized bronchi. Airway hyperresponsiveness, which is an extreme narrowing of the airways in response to a trigger or allergen such pollen, cold air, intense odors, or dust, is a major component of the pathogenesis. As a result of airway smooth muscle remodeling, increased mucus secretion, airway edema, and smooth muscle contraction, the airways become narrower (GINA,2023).

An asthma attacks pass through two stages; the early phase and the late phase. In the early phase; plasma cells have been sensitized and start to realizing IgE antibodies. These antibodies respond to precise environmental triggers, then attach to mast cells and basophils with great affinity. The cytokines released from mast cells and ultimately degranulate once a risk factor or pollutant is inhaled. Prostaglandins, leukotrienes, and histamine are altogether released via mast cells. The mast cells in turn make the smooth muscle to contract, narrowing the airway (Liu *et al*.,1991).

Th2 lymphocytes contribute significantly to the maintenance of inflammation by producing a number of interleukins (IL-4, IL-5, IL-13) and granulocyte-macrophage colony-stimulating factor (GM-CSF) that aid cells connect with one another. Basophils and eosinophils are helped to survive by IL-3 and IL-5. IL-13 causes smooth muscle remodeling, hyperplasia and fibrosis (Zhu *et al.,*1999).

After a few hours the late phase will be start and causes inflammation and bronchoconstriction via localizing basophils, eosinophils, neutrophils,

T-helper, and memory T-cells in the direction of lungs. Mast cells remain essential in carrying late phase reactants (Clifford *et al.*2009). It is essential to know these two mechanisms in order to target treatment and reduce both bronchoconstriction and inflammation, depending on the severity of the disease. It's interesting to note that those with a narrower airway over time tend to have lasting diseases. There is a transient restriction of the airway accomplished by bronchoconstriction and inflammation, which makes breathing more difficult (Doeing *et al*.,2013).

One of the main characteristics of asthma is airway hyperresponsiveness, which is characterized by an extra-bronchoconstriction reaction, typically to several stimuli. There are many mechanisms that cause airway hyperresponsiveness. Mast cell histamine production is raised in certain hypotheses, and airway smooth muscle hypertrophy is also increased. Additionally, there is a rise in intracellular free calcium and vagal tone, which improve the contractility of airway smooth muscle cells even more (Doeing *et al.,*2013).

The severity of airway hyperresponsiveness is assessed using bronchial provocation tests (Brannan *et al.*,2012). This feature is clinically applicable since it increases the probability of developing and aggravating asthma from early stages into adulthood and is connected to a more loss in lung function (Chapman *et al.*, 2015). Consequently, asthma and hyperresponsiveness might be treated precisely and early. Together, these structures modify the lungs' compliance only a little bit, making breathing to some extent more difficult. It might become more challenging for someone to breathe regularly as a result of inflammation, granular white blood cells, exudate, and mucus filling the bronchiolar trees. The rise in the amount of epithelium, which results in a tightening of the smooth muscle layer and lamina reticular, will increase the number of myo-fibroblasts, that produce collagen (Kudo *et al.,*2013). Subsequently the

basement membrane will be thickening more than normal as a result. A person's capability to breathe might be become permanently diminished, which is assumed to be the result of airway remodeling (Sinyor *et al.* 2022).

Remodeling happens when epithelial cells change from mesenchymal to smooth muscle. When epithelial cells come across tight junctions, they lose their cell adhesion and functional polarity and reformat into mesenchymal cells (Kudo *et al.,* 2013).

1.6. Mild and moderate asthma

Mild persistent asthma is defined as patients who experience asthmatic symptoms at least twice a week but less frequently than once per day. Patients over the age of five are diagnosed as mild persistent asthma if have PEF as well as FEV1 > 80% with variables 20-30% are qualified. Patients with moderate persistent asthma are those who experience daily asthmatic symptoms linked to exercise induced aggravation. For patients older than 5 years old, moderate persistent asthma is characterized by a PEF or FEV 1 that is within 60% to 80% of the expected values and a variability of greater than 30% (Yang, 2005).

1.7. Asthma diagnosis

Childhood asthma is typically diagnosed based on clinical manifestations, although it occasionally necessitates laboratory testing for atopy or hyperresponsiveness (including skin test, serum total IgE level, eosinophils count) in addition to pulmonary function test in younger or older children with concealed asthma symptoms (Shipp *et al.*, 2023).

1.7.1 Clinical diagnosis

The detailed history of intermittent wheezing, chest rigidity, coughing, and shortness of breath is the main concept for the diagnosis of asthma. It's possible that these episodes get worse with the passing of the seasons (such as in the spring or the late summer and early fall), or when

people exercise. It is essential to evaluate the history of nocturnal symptoms that result in morning awakenings. History of symptoms following exposure to other known triggers, such as cats, perfume, or cigarette smoking, is also extremely common. Significant factors include a favorable family history, rhinitis, or atopic dermatitis. Skin testing may be helpful in identifying allergens that cause asthma attacks after a thorough history has been taken, but it is only supportive in the diagnosis of the disease (Zeind *et al.* ,2018).

In order to increase global asthma awareness, prevention, and management, the World Health Organizations and US National Heart, Lung, and Blood Institute founded the Global Initiative for Asthma (GINA) in 1993. According to GINA guidelines asthma is more expected to be present in patients who show the following characteristics, which are classic symptoms of asthma: (Levy *et al,.*2006; GINA,2023)

1. The symptoms that patients (particularly adults) encounter is not limited to just one of these categories.
2. Wheezing, breathlessness, coughing, and/or tightness in the chest are respiratory symptoms.
3. In the early morning or evening, symptoms are frequently worse.
4. Intensity and duration of symptoms change throughout time.
5. Viral infections (colds), physical activity, exposure to allergens, changes in the weather, coughing, or irritants like smoke, strong odors, or exhaust fumes from moving vehicles can all cause symptoms to appear.

Asthma is less likely to be the cause of respiratory symptoms when the following characteristics are present (GINA,2023).

6. A cough without any additional respiratory symptoms.
7. Regular sputum production.
8. Breathlessness accompanied by lightheadedness, tingling in the periphery, or dizziness (paresthesia).
9. Chest discomfort.

10. Noisy inspiration caused by dyspnea during exercise.

1.7.2. Skin test

Immunoglobulin E has a cytotropic effect and attach to the high affinity IgE receptors on mast cells, so an intradermic injection of exogenous allergen extracts would cross link the cell-associated IgE and the high affinity IgE receptors on mast cells, causing in a hypersensitivity reaction within only fifteen minutes. In spite of its lack of specificity, this skin test is sensitive. This skin test might produce false-negative findings in children under the age of three or in those using anti-histamines (Shipp *et al*.,2023).

1.7.3 Serum Total Immunoglobulin E

IgE is an antibody synthesized by plasma cells in response to an antigenic stimulus; it induces type 1 hypersensitivity reactions and plays a critical role in the pathogenesis of allergic asthma. In mast cells and basophils, it binds to IgE receptors to produce cytokines that mediate T2 responses, which are characteristic of allergic asthma. Based on this, the role of IgE and the early sensitization of its relationship with asthma have been extensively studied (Romero-Tapia *et al.*, 2023). Serum Total Immunoglobulin E (IgE) test can be used as a primary screening for atopy since total IgE levels in serum can be determined through nephelometry or radioimmunoassay. Analyses of specific IgE by radioallergosorbent test, chemiluminescence mediated specific IgE, and fluoroallergosorbent are now commercially accessible and have sensitivity and specificity that are comparable to in vivo skin testing. Today, the best test to determine atopy is an allergy skin test. An in vitro blood IgE test is used for children who have inadequate skin responsiveness to an allergen test or who might be afraid of needle injections (Shipp *et al*.,2023).

1.7.4. Eosinophils count

Eosinophil levels in the blood and sputum can be increased in both allergic and nonallergic asthma, and this can be used to diagnose eosinophilic asthma. Serum eosinophilia can be used as a substitute marker for sputum eosinophilia, despite the fact that the latter is more accurate (de Groot *et al.,*2015). Sputum eosinophil count can be utilized to direct therapy in asthmatics, according to a metanalysis published in 2018 (Petsky *et al.*, (2018). Peripheral eosinophilia is linked to an increased frequency of asthma attacks in children. Serum eosinophils count is the asthma biomarker with the highest level of specificity (Tran *et al*.,2014).

1.7.5. Pulmonary Function Test (PFT)

Pulmonary disorders can alter the amount of air that can be breathe in and breathe out, so lung volumes are frequently evaluated to determine the size of the patient's lungs. The volume of inspired or exhaled air during typical breathing is known as tidal volume. The vital capacity is the amount of air exhaled from maximum inspiration (VC). The amount of air that remain in the lung following maximal expiration is known as the residual volume (RV). The functional residual capacity can be defined as the amount of air that remains after a typical expiration (FRC) and the sum of VC and RV is known as total lung capacity (TLC). Ventilation irregularities are made worse by forced expiratory volume. The forced expiratory volume (FEV) is the one and only test for a dysfunctional ventilator (Zeind *et al,*2018). The forced vital capacity (FVC) that defined as maximum volume of air exhaled with a maximally forced effort from a maximal inhalation, is frequently evaluated to assess the dynamics function of the lung in moving air. The ratio of FEV1/FVC is a common method to describe the FEV1 as a proportion of the total amount of air expelled. In general, healthy individuals can exhale at least 75% to 80% of their VC in 1 second and nearly all of it

in 3 seconds. As a result, the FEV1 is typically 80% of FVC. Lung volumes vary with age, race, sex, height, and weight; thus, it is necessary to compare the patient's breathing capacity to "predicted normal" values for patients with comparable physiologic features (Zeind et al. ,2018).

Peak expiratory flow (PEF) describes the maximal expiratory flow rate and expressed in liters/minute. The PEF can be easily measured with a variety of handheld peak flow meters, and it is frequently used in emergency departments and health center to promptly and quantitatively evaluate the efficacy of bronchodilators in the treatment of acute asthma episodes. The PEF is a less reliable measurement than the FEV1, however the changes in PEF usually correlate with those in FEV1. Effective asthma drug treatment reflected in both an improvement in absolute PEF values and a considerable reduction in diurnal variability. Diurnal variation of peak flow characterized by a morning fall in PEF, which is exaggerated in unstable asthma (National Bethesda MD,2007).

The FEV1 or PEF measurement are used as a critical guideline for step-up or step-down medicine for childhood asthma that ages older than five years. However, some studies questioned whether the PEF measurement might not even be essential to the managing of asthma and might not follow the guideline (Shipp *et al*., 2023). The Global Initiative for Asthma (GINA) has established specific recommendations for spirometry parameters including forced expiratory volume in 1 s (FEV1) and peak expiratory flow (PEF), which serve as indicators of large airway function and are used in the assessment and management of asthma (GINA ,2023).

Small airways are defined as the 7th-8th generation airways with an internal diameter of less than 2 mm with no cartilage in their walls, have been identified as the main sites of type 2 inflammation and airway remodelling in both adults and children with asthma. Small airway dysfunction has been associated with worse asthma control, increased

exacerbations, airway inflammation and airway hyperresponsiveness, increased risk for asthma development, and loss of lung function with aging in children (Yi et al.,2023).

The patient is asked to exhale into the spirometer as strongly and fully as they can after taking in their maximum amount of air. This is how the FEV is calculated. To determine the expiratory flow, the resulting volume curve is plotted versus time (Figure 1-1). Pneumotachographs in the mouthpieces of conventional spirometers, can detect airflow directly. The generated flow volume curves allow for the determination of several significant lung function measurements. The benefits of this technique include an investigation of the distribution of flow limitation, a prescription of concurrent flows at any lung capacity, a graphic evaluation of patient effort and collaboration, good reproducibility both within and across individuals (Zeind *et al.* ,2018).

Spirometry is the most commonly used and available lung function test. Spirometry parameters are influenced by weight, height, age, sex, ethnicity, environmental factors, patient cooperation and effort, and technical factors. The test can be performed easily in children older than six years of age. A few studies have also been shown to produce technically satisfactory results in preschool children (Jat et al.,2023).

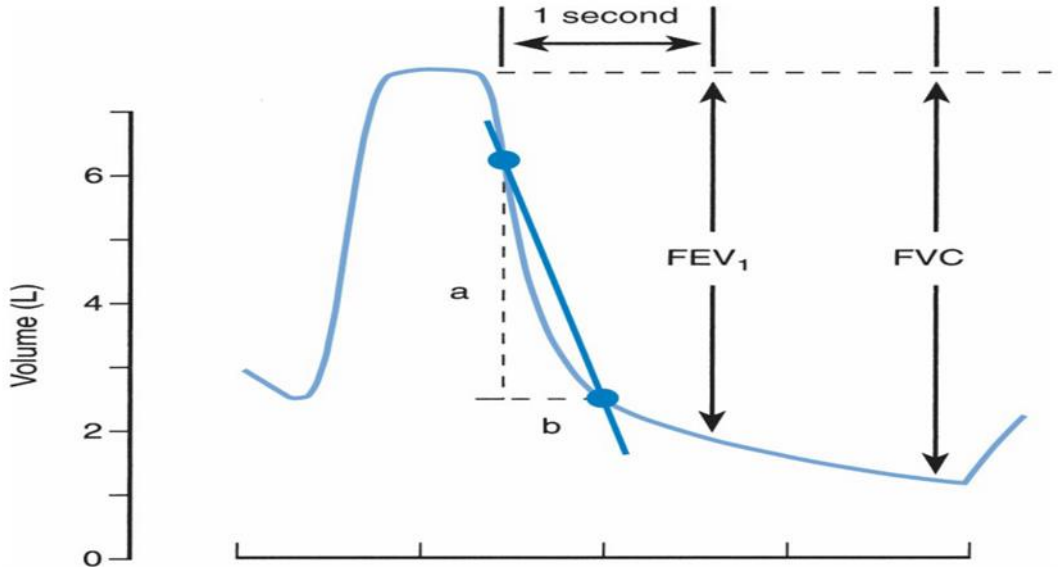


Figure (1-1) Volume–Time Curve from a Forced Expiratory Manoeuvre. FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity (Zeind et al. ,2018).

1.8. Asthma treatment

1.8.1. Goals of asthma treatment

The goals of asthma management in children are: controlling asthma symptoms, maintaining regular physical activity levels, keeping respiratory function, and preventing asthma attacks (Bahmani *et al*.,2023). Chronic airway inflammation caused by asthma may, over time, cause airway remodeling and pulmonary function loss that may not fully be reversed with therapy. Even before symptoms arise or an asthma diagnosis is obtained, the reticular basement membrane may thicken due to airway remodeling and injury. This emphasizes the value of early intervention and therapy (Boehmer *et al.*, 2006).

1.8.2. Main anti-asthmatic drugs classes

1.8.2.1. Inhaled corticosteroids (ICSs)

The best asthma control drug for children of all ages is inhaled corticosteroids (Bleecker *et al.*, 2020). Their effectiveness as a preventive therapy is explained by their broad impact on the inflammatory process. Binding to steroid receptors, which control the production of inflammatory

genes, mediates the anti-inflammatory effects. The production of cytokines and the secretion of inflammatory mediators are suppressed, as well as the migration and activation of inflammatory cells (Hossny *et al*.,2016; Gao *et al*.,2023).

Corticosteroids also limit the late-phase reactivity to allergen. The clinical benefits of ICS therapy include a decrease in symptom severity, an increase in asthma control as well as quality of life, an improvement in lung function, a decrease in airway hyperresponsiveness, the prevention of exacerbations, a decrease for systemic corticosteroid courses, and a decrease in emergency room visit, hospital stays, and asthma related deaths (Ye *et al.*,2017).

Asthmatic symptoms typically reappear when medication is stopped since using ICS does not provide remission of the condition. After stopping ICS, asthma control typically deteriorated within months (Chong *et al.*,2015).

1.8.2.2. Inhaled Short-Acting β2-Agonists (SABAs)

The Short-Acting β2 Adrenergic Agonists (SABA), called bronchodilators, are used to treat difficulties that occur while taking daily regulator drugs or during an exacerbation. After using it for five minutes, the bronchodilator effects reach their peak after 1 hour, and they can last up to six hours (Saini *et al.*,2023). The majority of sympathomimetic medications currently utilized to treat the bronchoconstriction associated with asthma are β_2-selective adrenoceptor agonists, mainly albuterol. Metered-dose inhalers are offered for the drugs pirbuterol, albuterol, terbutaline, and metaproterenol. These substances may be diluted in saline and administered using a portable nebulizer. Nebulizers produce substantially larger particles than metered dose inhalers do, requiring the administration of much higher doses (2.5-5.0 mg vs. 100-400 μg), which are not any more effective (Katzung *et al.* ,2012). Thus, patients who are unable to control their

breathing when using a metered dose inhaler should only get nebulized therapy (Nannini *et al.*,2021).

1.8.2.3. Inhaled Long-acting β2-agonists

Formoterol and salmeterol are examples of inhaled long acting β_2-agonists. By targeting specific β_2-receptors, β_2-agonists reduce the activity of the myosin light-chain kinase that is necessary for smooth muscle contractions. This results in the relaxation of airway smooth muscles. β_2-agonist not only dilate the airways but also stabilize mast cells. LABAs have a minimum 12 hours bronchodilation duration after a single dose because of their higher lipophilicity and extended retention in lung tissue. Salmeterol takes an hour after administration to reach its maximum bronchodilatation, but formoterol takes only five to ten minutes to start working (Jacobson *et al.,*2018).

For long term symptoms control and prevention among moderate to severe persistent asthma, inhaled LABAs are predominantly utilized as additional treatment in children under the age of 5 when combined with inhaled corticosteroids (alternative adjunctive therapy) or as a single-dose treatment before strenuous exercise. LABAs should not be used to treat severe asthmatic episodes. On the usage of LABA among children under the age of five, few data are known. Only children under the age of four can utilize salmeterol plus ICS with clinically beneficial results and safety. After a single dose, the LABAs efficiently block exercise indue bronchospasm (EIB) for 12 hours; but during long-term regular administration, this action is limited to 5 hours (Chauhan *et al.,*2015).

1.8.2.4. Anticholinergic drugs

Due to its low risk of side effects and accessibility for both metered dose inhaler MDI a nebulizer formulation, ipratropium has become the anticholinergic preferred choice for treating children. It relaxes the smooth

muscles of the airways by inhibiting muscarinic (M_3) cholinergic receptors. It decreases mucus production, minimizes mucous production, and prevents reflex bronchoconstriction brought on by irritants (Gosens *et al*.,2018).

Ipratropium inhalation therapy is typically used to treat acutely severe asthma. Additionally, those who cannot take SABA may utilize it as a substitute bronchodilator. Ipratropium, when combined with salbutamol, can enhance function and lower the likelihood of hospitalization in children who visit the emergency room with moderate to severe asthma attacks (Murphy *et al*.,2020).

Adverse effects include increased thirst, dry mouth and respiratory tracts, and some people ‘s wheezing and visual impairment if sprayed in the eyes. Ipratropium causes less cardiac stimulation during exacerbations than SABAs (Wyatt *et al.,*2015).

1.8.2.5. Leukotrienes Receptors Antagonist

Leukotriene modifiers, such as leukotriene receptor antagonist (LTRA) and a 5-lipoxygenase inhibitor, are offered as oral controller medications for the management of pediatrics asthma (Lee *et.al*.,2020).

There are two types of cysteinyl leukotriene 1 receptor antagonists: montelukast and zafirlukast. Patients over the age of 12 can get zileuton, an inhibitor of 5-lipoxygenase (Meshram *et al*.,2020).

1.9. Leukotrienes biosynthesis

Leukotrienes (LTs) are a group of potent bioactive lipid mediators synthesized from arachidonic acid (AA) through consecutive enzymatic steps by 5-lipoxygenase (5-LOX) and leukotriene A4 hydrolase (LTA4H) or leukotriene C4 synthase (LTC4S). Leukotrienes include a series of proinflammatory lipids mediator in which can become generated from arachidonic acid (AA) through a variety of cells that including mast cells, eosinophil, neutrophils, basophils, and macrophages and act in an autocrine

or paracrine manner, exerting their functions through the corresponding receptors on target cells. These where primary investigates in the 1970s (Peters-Golden *et al*.,2006; Teder *et al.,* 2023).

When the cell membrane is stimulated or injured, phospholipase A_2 catalases the degradation of AA from its phospholipids (Figure 1-2). The stimulation of AA hydrolysis from cell membrane phospholipids is shown in Figure (1-2). Platelet activating factor, calcium ionophore, or acute hyper-sensitivity are just a few examples of the immunologic or inflammatory processes that could be stimulated. The released free AA will then be separated from calcium ionophore or platelet activating factor. After being released into the free state, the free AA will be dehydrated by 5-lipoxygenase (5-LO) in conjunction with 5-lipooxygenase activating protein (FLAP) and turn into the unstable epoxide leukotriene A4(LTA4) (Ophir, *et al*,1985; Peters-Golden *et al*.,2003). Next, the unstable epoxide LTA4 is transformed into leukotriene B4 (LTB4) by the enzyme LTA4 hydrolase, and then into LTC4 by the enzyme leukotriene C4 (LTC4) synthase (Haeggström *et al.*, 2004).

LTC4 will be transformed to leukotriene D4 (LTD4) after being transported into the extracellular environment. Last but not least, dipeptidase removes the glycine residue from LTD4 and converts leukotrienes E4 (LTE4) (Al-Azzam *et al*., 2020).

LTB4 was not categorised as cysteinyl leukotriene because it lacked the peptide associated chain that these compounds possess (Crooks *et al.,*1998).

As a conventional chemoattractant, LTB4 could cause leukocytes to aggregate and attach to the endothelium. Additionally, it controls the immunological reactions linked to host defense against pathogens. Numerous inflammatory disorders, including as nephritis, chronic

obstructive pulmonary disease, dermatitis, and arthritis, are impacted by LTB4 (Haeggström *et al,* 2004).

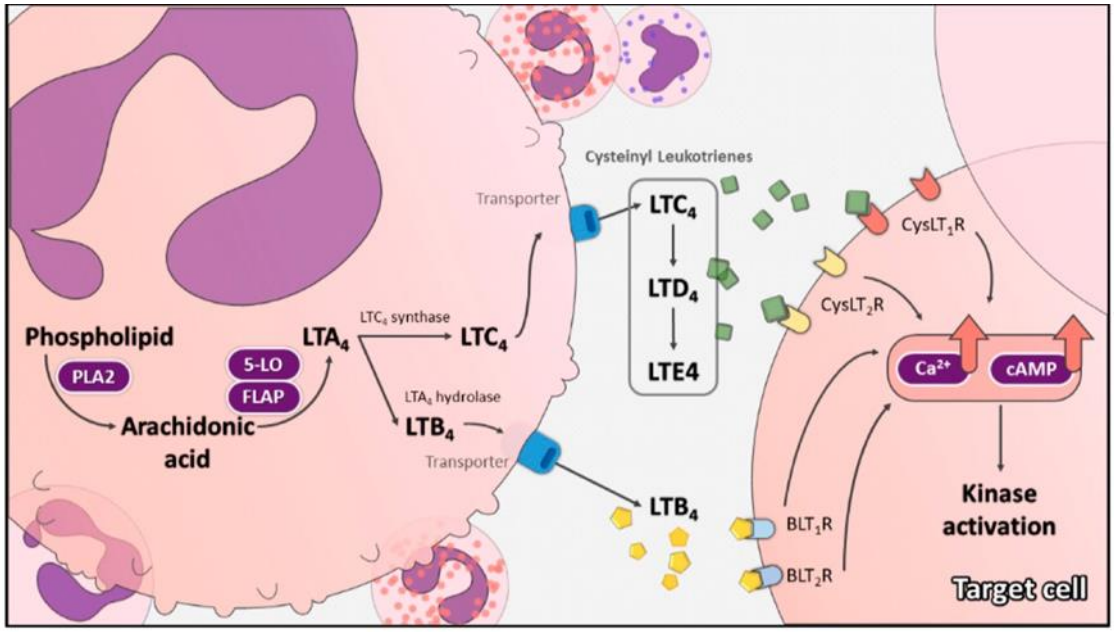


Figure (1-2): The Paths of Cysteinyl Leukotriene. LTC4 synthase changes leukotriene A4 (LTA4), which comes from arachidonic acid, into leukotriene C4 (LTC4). LTC4 is transformed into leukotriene E4 (LTE4) and leukotriene D4 (LTD4) after entering the extracellular environment. LTC4, LTD4, and LTE4 are examples of cysteinyl leukotrienes. These substances bind to CysLT1R and CysLT2R to activate the signaling pathways in the target cells (Al-Azzam, et al., 2020).

1.9.1. Cysteinyl Leukotrienes

Figure (1-2) shows the cysteinyl leukotrienes (CysLTs), which include LTC4, LTD4, and LTE4. The most stable of these, which may be detected in the urine, is LTE4. Therefore, the urine LTE4 level could be utilized as a measure of "whole body" leukotriene production (Singh *et al*., 2010). In addition to their well-known role in inflammation, CysLTs have also been shown to exhibit pro-angiogenic properties (Tsopanoglou *et al*.,1994).

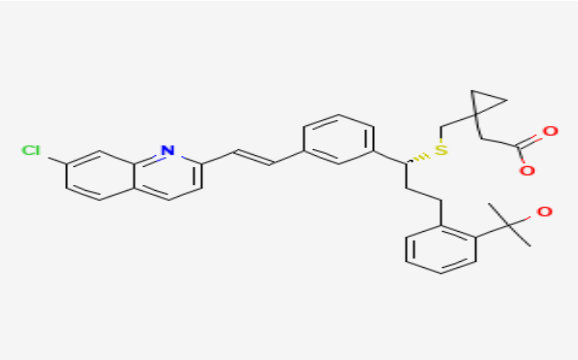
CysLTRs, also known as cysteinyl leukotriene receptors, are divided into three groups: CysLT1R, CysLT2R, and CysLT3R. Varied CysLTs have varied receptor affinities: for the CysLT1R, LTD4 is more affinity than

LTC4 and more affinity than LTE4; for the CysLT2R, LTD4 is affine but LTC4 is more affinity than LTE4 (Rovati *et al.*, 2007).

1.9.2. CysLT1 Receptor (CysLT1R)

Human tissues that have high concentrations of CysLT1R include brain, gastrointestinal tract, peripheral blood leukocytes, and respiratory system (Peters-Golden *et a. l*,2006). In the respiratory tract of asthmatic patients, CysLT1R may be expressed by cells of smooth muscle, epithelium cells, interstitial lung macrophages, and basophils (Ravasi *et al*., 2006; Profita *et al*.,2008). Additionally, peripheral blood leukocytes such as neutrophils, pregranulocytic $CD34^{+}$ cells, eosinophils, monocytes, macrophages, and certain B lymphocytes have been shown to express CysLT1R (Peters-Golden *et.al*,2006). Colon and brain tumors both express CysLT1R (Rovati *et al.,* 2007).

1.10. Montelukast



Empirical formula $C_{35}H_{35}C_{1}NNaO_{3}S$

Figure (1-3) Tthe chemical structure of montelukast (Al-Allaf *et al.,* 2023).

The presence of a chemical that promotes smooth muscle constriction in asthmatic patients' sputum has been recognized to researchers since 1930 (Lee *et al.,*2020). This substance was given the name slow reacting substance of anaphylaxis (SRS-A) because, when released from sensitized lungs during an allergic reaction, it induces delayed contractions of the smooth muscle (Moon *et al*., 2009). SRS-A was discovered to belong to the LT family in the late 1970s (Murphy *et al.,*2018). In isolated human

bronchi, the LTs C4 and D4 apparently demonstrated considerable contractile activity in studies done in 1980. Following that, there were several studies looking at LTs as potential asthma therapies. LTRAs (leukotriene receptor antagonists) and 5-LO (5- lipoxygenase inhibitors are examples of LT modifiers, Zafirlukast, montelukast, and pranlukast is examples of LTRAs, whereas zileuton is a 5-LO inhibitor. The most frequently prescribed and researched of these is montelukast (Kolmert *et.al*,2021).

1.10.1. Pharmacokinetics and pharmacogenetics of montelukast

Montelukast is immediately absorbed after being administered orally . There is a 64% mean oral bioavailability in adults who take 10 mg of montelukast tablet. Montelukast is 99% bound to plasma proteins, according to distribution (Narmetova,2024). The liver is the main site of metabolism for montelukast. At extents that are clinically significant, CYP2C8 appears to be quite significant in the montelukast metabolism. Montelukast and its metabolites are eliminated almost exclusively through the bile. At dosages up to 50 mg, montelukast's pharmacokinetics are linear. No dosage adjustment needed in patients with mild to moderate hepatic insufficiency. The pharmacokinetics of montelukast in patients with more severe hepatic impairment have not been assessed according to product labeling. No dosage adjustment recommended in patients with renal insufficiency because it excreted via the bile (Zaid *et al* ,2015; Wermuth *et al*., 2023).

A previous study revealed that patients having the variant rs12422149 in SLCO2B1 gene encoding the solute carrier organic anion transporter family member 2B1 that mediates montelukast permeability have reduced montelukast morning plasma concentration after an evening dose and no

symptoms improvements after montelukast use observed in these patients (Mougey EB et al., 2009). Other studies have demonstrated that polymorphisms in genes encoding transporter protein MRP1, ALOX5, ALOX5 tandem repeat promoter polymorphism and the LTC4S contribute to variability in response to LT receptor antagonists (Lima JJ et al., 2006; Lima JJ, 2007). rs6475448 SNP reported a novel association with an improvement in response to montelukast in four independent asthmatic populations, rs6475448 SNP present within MLLT3, which regulated cell fates for megakaryocytes and early erythroid cells in humans (Dahlin, *et al.*,2015).

1.10.2. Mechanism of action of montelukast

Montelukast, a leukotriene receptor antagonist, bind with high affinity to the cysteinyl leukotriene receptor of leukotrienes D4 and E4. The D4 and E4 leukotrienes contribute to the inflammation that might cause in the signs and symptoms of asthma and allergic rhinitis. These leukotrienes are excreted by a variety of cells, including mast cells. Airway cells including macrophage and smooth muscle cells contain leukotriene receptors. Montelukast does not demonstrate any agonist activity when attached to leukotrienes receptors, but instead suppresses leukotriene physiological properties (for example; edema in the airway, contraction of smooth muscle, and disturbance of usual cellular action). The leukotriene D4, induced bronchoconstriction is significantly inhibited by low dosages of montelukast (5mg) in asthmatics. Also, montelukast caused suppression of bronchoconstriction in both early and late phase that brought on by an antigen challenge (Castro-Rodriguez *et al.,*2018; Al-Allaf *et al*.,2023).

Montelukast has been shown to considerably increase morning peak expiratory flow and significantly decrease β2-agonist use, asthma symptoms, and all three. These measures that montelukast reduces

eosinophilic inflammation in the airways and enhances clinical symptoms. Its impact on airway inflammation may play a role in its ability to effectively treat chronic asthma (Ramsay *et al*.,2009).

1.10.3. Indications

Montelukast usually prescribed for following condition:

Asthma: Leukotriene receptors antagonist (LTRAs) are suggested as monotherapy for mild persistent asthma by the Global Initiative for Asthma (GINA) recommendations as an addition to or substitute for increasing the dose of inhaled corticosteroids (ICS) or using a long-acting $\beta_2$agonist (LABA) (Miligkos *et al*.,2015). Montelukast is approved for both acute and chronic therapy of asthma in adults and children 12 months of age and older (Sánchez *et al*., 2018; Sun *et al*. 2019, Jafari *et al*.,2023).

Exercise Induced Bronchoconstriction (EIB): Patients with six years of old and older who are taking montelukast are at reduced risk of developing exercise-induced bronchoconstriction (EIB) (De Benedictis *et al.,*2008).

Seasonal Allergic Rhinitis: Patients that have two years old and older, as well as those six months of age and older, should take montelukast to relieve the symptoms of seasonal allergic rhinitis. Montelukast should only use in individuals who have poor response and/or intolerance to alternative medications since the advantages might not be outweigh the risk of neuropsychiatric adverse events in persons having allergic rhinitis. The recommended oral dose of montelukast is listed in table (1-1) (Krishnamoorthy *et al.,*2020; Ghimire *et al.,*2023).

Table (1-1): Recommended dose of montelukast

Ages	Dosage Regiment
15 years old and older	10 mg
6-14 years old	5 mg
6 months to 5 years old	4 mg

1.9.4. Adverse effects

Despite being conventionally regarded as safe and possessing well-described anti-inflammatory properties and bronchoprotective actions (Straub *et.al*,2005), montelukast may cause adverse events, most of which are moderate (Forrester *et al.,*2007). Numerous studies have focused in particular on the prevalence of neuropsychiatric side effects, which can include everything from nightmares and sleep disturbances to aggressive behavior, nervousness, and suicide thoughts (Paljarvi *et al,*2022; Maglion *et al*., 2023; Lo *et al*., 2023).

These symptoms often start within the first 10 days of the first administration and are more prevalent in children aged 4 to 6 years (Watson *et al*,2022). Even though they are less frequently recorded, adverse events following montelukast therapy may affect a number of other organs and systems. Even though the majority of them had been seen in adults, an Italian assessment of published case reports included an overview of the possibly hazardous effects linked to its administration (Dixon *et al*,2021).

Montelukast administration may be associated with gastrointestinal symptoms as vomiting, nausea, and stomach discomfort (Bian *et.al* ,2021), and an incident of hepatic toxicity in five years old child has been seen (Incecik *et.al* ,2007; Calapai *et.al*,2014).

1.10.5. Toxicity

High doses of montelukast (up to 200 mg per day for 22 weeks and up to 900 mg\day for a week) that have been administered to adult patients, don’t show any unfavorable side effects. Montelukast overdose cases involving doses as high as 1000 mg have been documented in both adults and children. Clinical and biological symptoms in such cases, however,

typically included thirst, headaches, drowsiness or hyperactivity, vomiting, and abdominal disturbance (Lassila *et al*,2015).

There are no known mutagenic or carcinogenic effects, of montelukast, however, this chemical has not been linked to any teratogenic or fertility-related side effects (Wermuth *et al.,*2023).

1.11. Genetics polymorphism

The most prevalent types of genetic variation are Single nucleotide polymorphisms (SNPs) that exists at a certain nucleotide location in different people and alters the pharmacokinetics and pharmacodynamic parameters of drugs (Ismail et al. 2012). There are two categories of those who fall into the coding regions: synonymous or silent and non-synonymous (Hunt et al.,2009).

1.12. Organic Anion Transporting Polypeptides (OATP)

Human Organic Anion Transporting Polypeptides (OATPs; SLC gene family SLC21/SLCO) are significant uptake transporters that are express in a variety of tissues, including the blood brain barrier, liver, kidney, and placenta (Hoste *et al.*, 2023). Genetic polymorphisms in SLCO2B1 and ABCC1 conjointly modulate atorvastatin intracellular accumulation in HEK293 recombinant cell lines. Eleven of the human OATP members family have now been identified and classified into six distinct subfamilies (OATP1-OATP6). A wide variety of amphiphilic organic anions are transported into cells by them through a sodium-independent process. Endogenous substances like hormones or their metabolites, bile salts, and xenobiotics, including several commonly given medications like statins, cardiovascular medications, and antibiotics, are examples of substrates for human OATPs (Kindla *et al*.,2011; Li *et al*., 2023).

OATP2B1 has been demonstrated to have a wide tissue distribution, and in addition to above organs, it also detected in skeletal muscle, kidney, and small intestine, in contrast to OATP1B1 and OATP1B3, which are typically thought of as liver specific (Kim *et al*.,2017).

The first description of Organic Anion Transporting Polypeptide (OATP) 2B1 was made in 2000. The transporter was identified as OATP-B in the initial study and was demonstrated to be widely expressed by the extreme mRNA levels in the newborn and liver of adult (Kinzi *et al*.,2021).

OATP2B1 seems to be a glycoprotein with 709 amino acid that is encoded by the SLCO2B1 gene and has 12 presumed membrane spanning loops. OATP2B1 is extensively distributed in humans and is being found in a variety of healthy tissues. Despite the fact that the transporter was first discovered in the brain, the tissue exhibits very modest levels of expression as comparing either to liver and other tissues. OATP2B1 is thought to aid in absorption of substances into practically all tissues. OATP2B1 could be expressed on the basolateral membrane of enterocytes in the intestine, according to some researchers. For example, Mooij, identified the transporter as aiding compound transfer from the enterocyte to blood, in contrast to Keiser, who identified the transport as having occurred from the blood circulation into the enterocyte (McFeely *et al*.,2019)

The paradoxical results suppose that OATP2B1 may serve a regulatory action in eliminating bile acids and hazardous metabolites from the circulation, in addition to its traditional role in chemical absorption. About 9% of the liver's total measured transporter protein is OATP2B1. OATP2B1 is about half as abundant as OATP1B1, but about equally abundant as OATP1B3. This result was verified for healthy Caucasian patients by a meta-analysis of transporter abundance data (McFeely *et al*.,2019).

1.12.1. OATP2B1 genetic polymorphism

Drug transporters are expressed on the plasma membrane and determine the pharmacokinetics of various drugs. The activity of the transporter is affected by various factors, such as genetic variations and post-translational modifications (Yajima *et al.,* 2023). In the case of OATP2B1, several nucleotide substations have been documented, including: rs2306168 (c.1457, C>T; p. Ser486Phe), rs1621378(c.1175, C>T; p. Thr392Asn), rs35199625 (c.601, G>A; p. Val201Met), rs12422149 (c.935G>A) (figure 1-4). Yet, most of these polymorphisms seem to be more common in specific populations (for example, Koreans and Japanese) and have thus been examined in clinical investigations. The rs12422149 polymorphism that results in an arginine to glutamine substitution at position 312 has been explored the most studied polymorphism for montelukast (Mougey *et al*.,2011).

Studies conducted in vitro have yielded inconsistent outcomes regarding genetic variations effects on the transport activity of OATP2B1. OATP2B1 and OATP1A2 were found to be the two transporters involved in the absorption of montelukast by Mougey *et al*., (2009). Although there is significant experimental support for the relevance of intestinal OATP2B1 to drug absorption (McFeely et al., 2019), the impact of this transporter on drug distribution and elimination in other tissues where it is also expressed, remains significantly less understood (Kinzi et al., 2021).

The genetics polymorphism of OATP2B1 rs12422149 (G > A), that results in the substitution of amino acid R312Q has been connected to montelukast plasma level, according to these authors. They noticed that subject homozygotes had higher plasma concentrations than heterozygotes. In addition, compared to baseline scores, the Utility Index scores of Asthma Symptom in homozygotes increased after one and 6 months of montelukast therapy. Therefore, the pharmacokinetics and therapeutic

response of montelukast may be impacted by the SNV rs12422149 (García-Menaya *et.al*,2019). The same authors confirmed these findings in a subsequent investigation on adolescents (Mougey *et al*., 2011). However, two studies on healthy White Finnish and Korean populations, separately, found no evidence to substantiate the effects of the OATP2B1 SNV rs12422149 on pharmacokinetics of montelukast (Kim *et al.*,2013; Tapaninen *et al.*,2013). The OATP2B1 c.935G>A variation has primarily been linked to decreased transportation activity, but its significant effect seems to be extremely depended on substrate and experimental model (Medwid *et al,*2021).

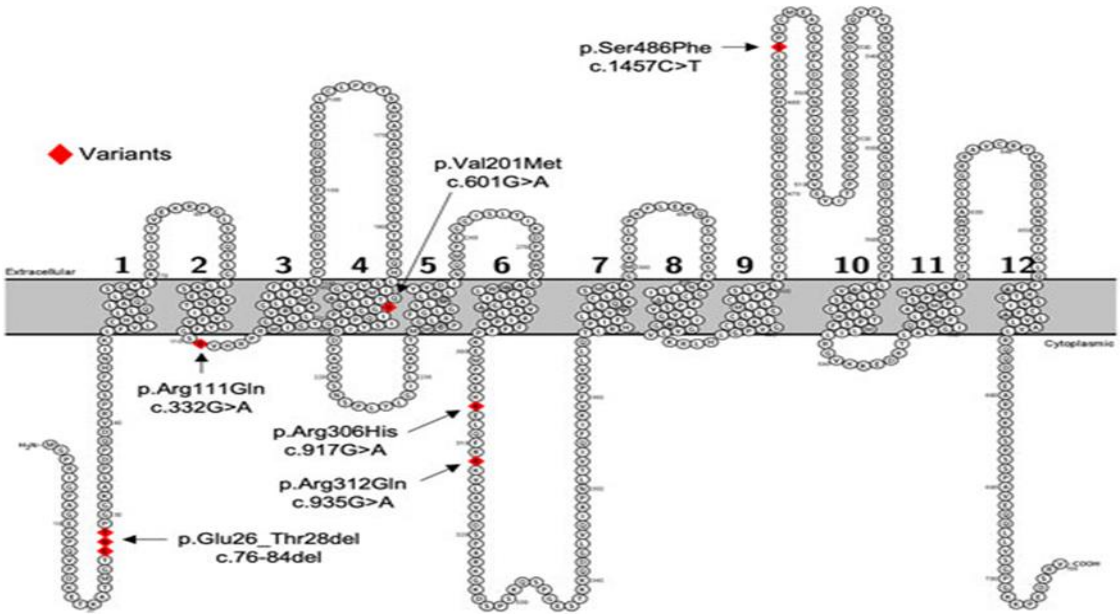


Figure (1-4) Predicated 2-D Structure of OATP2B1 Full Length Transcriptional Variant. Genetic polymorphisms of interest are painted in red and directed by arrows with residual number and amino acid variation. The predicted membrane topology 2-dimensional model of OATP2B1 was created via Protter interactive protein imagining software (Medwid et al.,2021).

1.13. Montelukast metabolism

Many metabolites of montelukast are excreted with bile representing for the large amounts and urine for less than 0.2%. There are three primary metabolites: a phenol(M3), diastereomeric 21-hydroxylated metabolites

(M5a and M5b), and diastereomeric methyl- hydroxylated metabolites (M6a and M6b) (Figure (1-5). The quantitatively most significant bile metabolite is a dicarboxylic acid metabolite (M4 and M4b), which is produced by continued oxidation on M6 (Filppula *et al*,2011).

Bile also contains traces of a diastereomeric sulfoxide (M2a and M2 b) and an acyl glucuronide metabolite (M1) (Filppula *et al.,*2011).

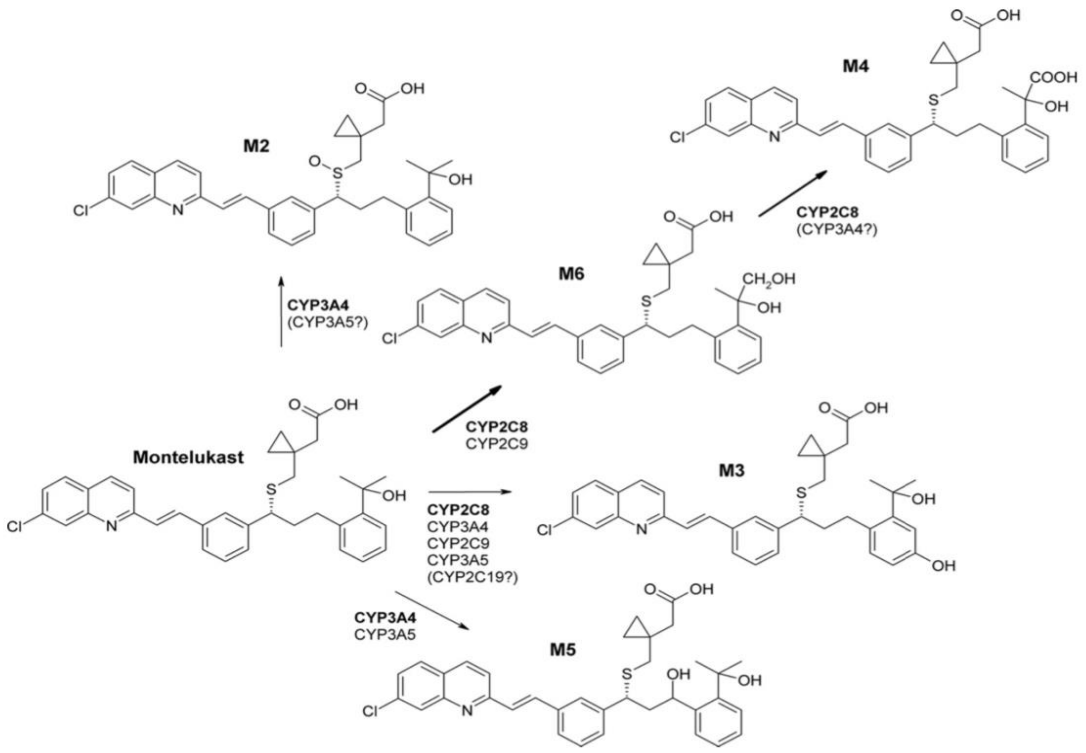


Figure (1-5). Montelukast Oxidative Metabolism. The isoforms of P450 are responsible for the development of the metabolites M2, M3, M4, M5, and M6 are shown according to Chiba et al. (1997) and Filppula work (Filppula, *et al.*,2011).

Montelukast clearance is mediated by a variety of distinct enzymes. The primary P450s involved in montelukast metabolism are listed as CYP2C9 and CYP3A4 in an initial in vitro experiment and on the product label; CYP2C9 was thought to be selective for montelukast 36-hydroxylation. Recent in vitro investigations, however, link CYP2C8 to36-hydroxylation of montelukast, however a further look at the in vitro

research, reveals that P450s other than CYP2C8 may also be implicated. Montelukast's capacity to firmly bind to the CYP2C8 active site and to strongly and competitively suppress this enzyme in vitro provide more evidence for the potential function of CYP2C8 (Cardoso, *et al.*,2015).

1.13 .1 Microsomal metabolizing enzyme CYP2C8

CYP2C is an important cytochrome P450 occurred in the liver, which plays an important role in the oxidation of both xenobiotic and endogenous compounds (Patil et al., 2023). The phase I metabolizing enzyme of cytochrome P450, family 2, sub family C, polypeptide 8 (CYP2C8) is essential for the biotransformation of a varied range of xenobiotics and endogenous chemicals. CYP2C8 makes up 7% of the liver's CYP content and is expressed to varying degrees in the brain, ovary, uterus, duodenum, adrenal gland, and mammary gland (Aquilante *et al*.,2013). Around 5% of medicines are oxidized by CYP2C8. This enzyme is critical for the clearance of medications used to treat diabetes (repaglinide, rosiglitazone, pioglitazone), malignancy (paclitaxel, trans retinoic acid, enzalutamide), anemia (daprodustat), parasites (amodiaquine and chloroquine), asthma (montelukast), viral HEPC (dasabuvir), arrhythmias (amiodarone), and statins (cerivastatin) (Desta *et al.*, 2017).

1.13.2 Genetic polymorphism of CYP2C8

The CYP2C8 gene, which is near to the CYP2C9 gene on chromosome 10q24's 2C gene cluster, codes for the CYP2C8 enzyme. The CYP2C8 gene, which spans a 31 kb length and has 9 exons, is the shortest of the human CYP2C genes (Backman *et al.*,2015). The CYP2C8 gene now contains around 100 non-single nucleotide polymorphisms (SNPs). The most prevalent of them are CYP2C8*2, CYP2C8*3, and CYP2C8*4. At an allelic frequency of 18%, the CYP2C8*2(Ile 269Phe in exon 5) allele is primarily seen in Africans. Caucasians are predominately carriers

of CYP2C8*3 (Arg139Lys and Lys399Arg in exons3 and 8; allelic frequency of 10-23%) and CYP2C8* 4 (Ile264 Met in exon 5; allelic frequency of 7.5-11%). Both of these alleles are relatively uncommon in Asians (0.5%). There are very few other variations that modify the amino acids. Moreover, two SNPs (*1B and *1C) in the regulatory regions of CYP2C8 have been discovered (Desta *et al.*,2017). The allele frequency for CYP2C8*1B (rs7909236) was only 8% in Chinese population which is lower than those of Caucasians (24%) (Li *et al*.,2019).

1.14. The aims of study

1. To study the distribution of different genotypes of OATP2B1 c.935G>A (rs12422149) transporter and CYP2C8*1B g.-271 (rs7909236) enzyme on in asthmatic children.

2. To investigate the effect of genetics polymorphism of OATP2B1 c.935G>A (rs12422149) transporter and CYP2C8*1B g.-271 (rs7909236) enzyme on montelukast therapeutics response in asthmatic children.

3. To investigate if there is any association between different genotypes of OATP2B1 c.935G>A (rs12422149) transporter and CYP2C8*1B g.-271 (rs7909236) enzyme and efficacy of montelukast therapy response in asthmatic children.

Chapter Two
Patients, Materials, and Method

Patients, Materials, and Methods

2.1. Study design and patients' selection

The observational cross-sectional study was conducted on 100 asthmatic children (male and female) aged 6 to 15 years at Kerbala Teaching Hospital for Children from end of October 2022 to the end of September 2023. Children with mild to moderate asthma were selected from Respiratory Clinic Centre in the Hospital after receiving written consent from their parents and filling in designed questionnaire after informing them of the nature and objectives of the study.

2.1.1 Inclusion criteria

The children who were included in this study must have the following criteria:

1. Mild to moderate asthmatic attacks.
2. Used montelukast (5mg and 10mg) regularly as monotherapy for at least one month and more.

2.1.2 Exclusion criteria

The children are excluded if they have one or more of the following criteria:

1. Patient with severe acute asthmatic attack.
2. Patient with history of chronic diseases like chronic cardiac disease, chronic renal failure, and diabetes mellitus.
3. Pneumonia, tuberculosis (TB) or other respiratory diseases, documented by chest x-ray (CXR).
4. Patient that taking any drug effect on OATP2B1 transporter and/or CYP2C8 enzyme activity.

2.2. Study protocol

In the present study, demographic parameters (age, gender, weight, height and body mass index (BMI)) were taken. The BMI calculated and

categorized from Centers for Disease Control and Prevention (https://www.cdc.gov/healthyweight/bmi/calculator.html). The response of asthmatic children to montelukast therapy was done clinically by evaluating lung function test using spirometry and asthma control test (ACT) after one month of montelukast therapy. Besides, measuring total serum IgE.

2.3. Ethical approval

The protocol of the study was approved by the ethical research and scientific committee of College of Pharmacy, University of Kerbala. Approval was also taken from Karbala Health Directorate and Administration of Karbala Teaching Hospital for children. In addition, consent was taken from parents of each patient after explaining the nature and purpose of study.

2.4. Materials

2.4.1. Chemicals and kits

All Chemicals and Kits used in this study are listed in table (2-1).

Table (2-1): Chemicals and Kits

Chemical and Kits	Manufacture	Country
Agarose powder	Condalab	Spain
Accupower® PCR PreMix Kit	Bioneer	Korea
DNA Ladder 100-3000 bp	SoloGent	Korea
Ethanol 90%	Honeywell	Holland
Ethidium Bromide solution	Marliju	Korea
Genomic DNA extraction kit	Macrogen	Taiwa
Montelukast (5mg and 10 mg)	Pioneer	Iraq
Nuclease free water	Bioneer	Korea
Primers	Macrogen	Korea

Tris borate EDTA (TBE) Buffer 10x	Marliju	Korea
Total Immunoglobulin E diagnostic kit	Euroimmun	German

2.4.2. Instruments

The instruments used in this study are described in table (2-2).

Table (2-2): Instruments

Instrument	Manufacture	Country
Autoclave	Incubator	Germany
Centrifuge	Sigma	England
Cold Medical Box	HEMC Medical	China
Digital Camera	Canon	China
Disposable turbine	MIR	Italy
EDTA and gel Tube	Shandong Yaoha	China
Eppendorf tube (2 ml)	Fisher Scientific	England
Freezer	Panasonic	Korea
Gel Electrophoresis system	Cleaver Scientific	England
Hotplate Stirrer	Lab TEch	Korea
Micropipettes (different size)	Gilson Pipette	Japan
Nano-drop	Bio Drop	England
PCR- thermocycler	TECHNE	England
Sensitive Balance	DEN-EVER	Germany
Spirometer (Spirolab III)	MIR	Italy
U.V Trans illuminator	Cleaver Science	England
Vortex mixer	Human Twist	Germany

2.5. Methods

2.5.1. Blood samples collection in asthmatic children

A blood sample (4 ml of venous blood) was collected from all asthmatic children. Two ml was used for genetic testing, while the second 2 ml were used to measure total IgE level.

2.5.2. Asthma control tools in asthmatic children

The asthma control tools used in this study include; Asthma control test (ACT) score and pulmonary function test (the only parameters FEV1 and PEF had been taken). These tools are routinely used in Kerbala respiratory Center to assess patient symptoms enhancement after taking montelukast and other controlled therapy in every month patient visit clinic. In this study the result of these tests were taken after at least four weeks of therapy.

2.5.2.1 Assessment of lung function in asthmatic children

Lung function was evaluated by spirometer (Spirolab I) using the forced vital capacity mode (FVC) and disposable turbine system.

2.5.2.1.1 Procedure

According to the standardization of spirometry by the American Thoracic Society (ATS) and European Respiratory Society (ERS) (Graham, *et al.*, 2019; Bhakta *et al*., 2023) the following protocol was adopted:

1. An easy explanation of the procedure was given to the child.
2. They were warned not to take the test while wearing anything constricting or tight.
3. The patient was either standing or seated during the test.
4. A nose clip was used to stop nasal exhalation or inhalation, and a new disposable mouthpiece (turbine) was fitted to the spirometer.
5. The patient was instructed to exhale normally while squeezing his lips tightly around the mouthpiece.

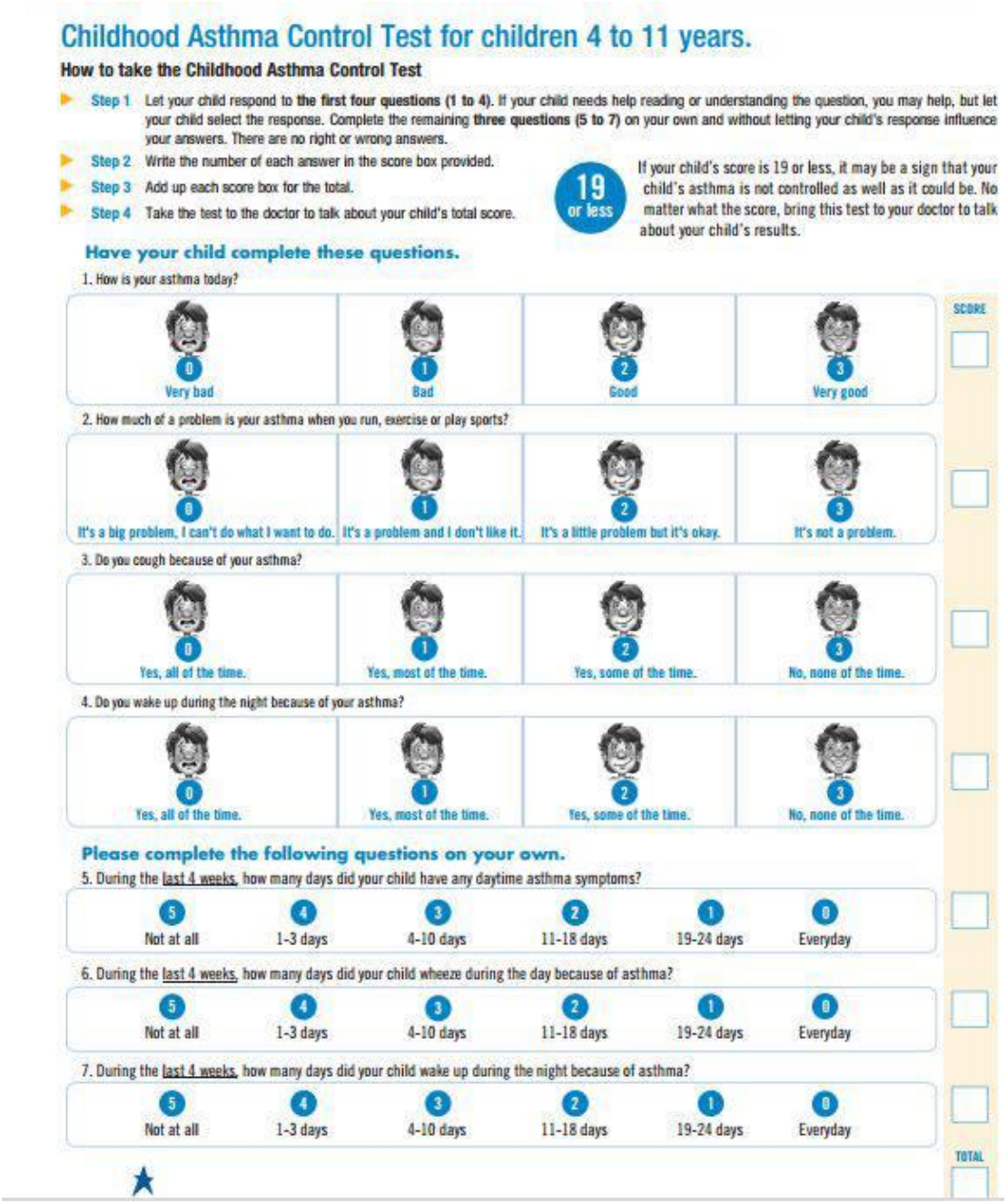
6. The final stage was a quick and powerful expiration after a slow maximal inhale to increase the inspiratory reserve capacity to its maximum.
7. As indicated by a plateau on the volume time graph the blow is prolonged until exhalation is complete with no pauses, interruptions from other breaths or coughs and no exhalation through the nose.
8. Children's participation in spirometry was encouraged via computer generated reward graphics.
9. For each evaluated child, these processes were repeated three times.

The highest score of the three trials was recorded, and the results were only reported if more than two technically acceptable curves were obtained after choosing the best one. The measured parameters for current study were;

1. FEV1 (Force Expiratory Volume in one second)
2. PEF (Peak Expiratory Flow)

2.5.2.2 Assessment of Asthma Control Test (ACT) score in asthmatic children

Asthma Control Test (ACT): The scores of asthma control are classified into two categories; controlled asthma; if patient's score ≥20 and uncontrolled asthma; if patient's score ≤19. The ACT has four symptom/ reliever questions plus patient self-assessed control. The minimum clinically important difference is 3 points (Schatz, *et al.,*2009).



Childhood Asthma Control Test for children 4 to 11 years.

How to take the Childhood Asthma Control Test

- **Step 1** Let your child respond to **the first four questions (1 to 4).** If your child needs help reading or understanding the question, you may help, but let your child select the response. Complete the remaining **three questions (5 to 7)** on your own and without letting your child's response influence your answers. There are no right or wrong answers.
- **Step 2** Write the number of each answer in the score box provided.
- **Step 3** Add up each score box for the total.
- **Step 4** Take the test to the doctor to talk about your child's total score.

19 or less If your child's score is 19 or less, it may be a sign that your child's asthma is not controlled as well as it could be. No matter what the score, bring this test to your doctor to talk about your child's results.

Have your child complete these questions.

1. How is your asthma today?

0	1	2	3	SCORE
Very bad	Bad	Good	Very good	

2. How much of a problem is your asthma when you run, exercise or play sports?

0	1	2	3	
It's a big problem, I can't do what I want to do.	It's a problem and I don't like it.	It's a little problem but it's okay.	It's not a problem.	

3. Do you cough because of your asthma?

0	1	2	3	
Yes, all of the time.	Yes, most of the time.	Yes, some of the time.	No, none of the time.	

4. Do you wake up during the night because of your asthma?

0	1	2	3	
Yes, all of the time.	Yes, most of the time.	Yes, some of the time.	No, none of the time.	

Please complete the following questions on your own.

5. During the last 4 weeks, how many days did your child have any daytime asthma symptoms?

5	4	3	2	1	0	
Not at all	1-3 days	4-10 days	11-18 days	19-24 days	Everyday	

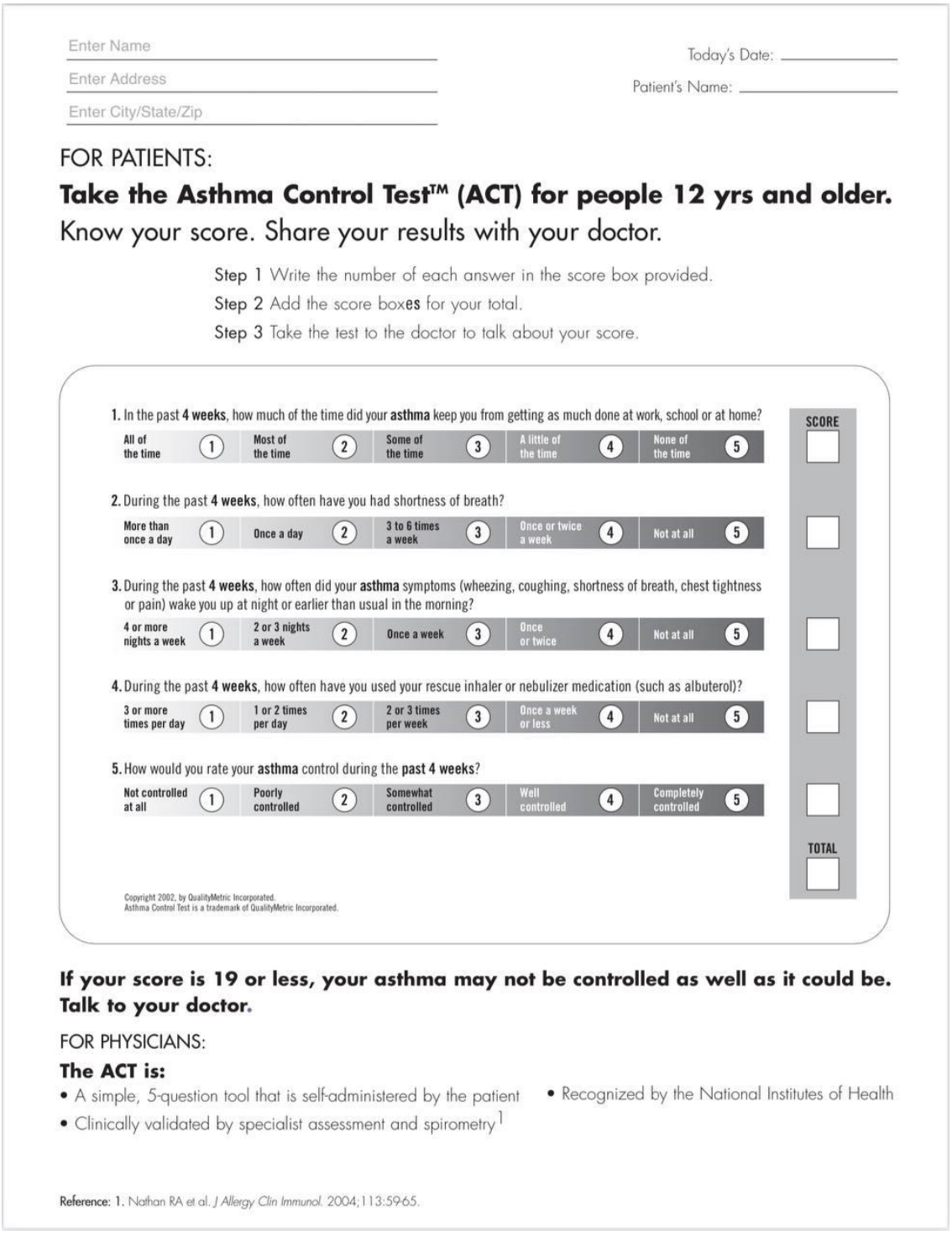
6. During the last 4 weeks, how many days did your child wheeze during the day because of asthma?

5	4	3	2	1	0	
Not at all	1-3 days	4-10 days	11-18 days	19-24 days	Everyday	

7. During the last 4 weeks, how many days did your child wake up during the night because of asthma?

5	4	3	2	1	0	
Not at all	1-3 days	4-10 days	11-18 days	19-24 days	Everyday	

TOTAL



Enter Name

Enter Address

Enter City/State/Zip

Today's Date: _____

Patient's Name: _____

FOR PATIENTS:

Take the Asthma Control Test™ (ACT) for people 12 yrs and older.

Know your score. Share your results with your doctor.

Step 1 Write the number of each answer in the score box provided.

Step 2 Add the score boxes for your total.

Step 3 Take the test to the doctor to talk about your score.

									SCORE
1. In the past **4 weeks**, how much of the time did your **asthma** keep you from getting as much done at work, school or at home?	All of the time	1	Most of the time	2	Some of the time	3	A little of the time	4	None of the time	5	
2. During the past **4 weeks**, how often have you had shortness of breath?	More than once a day	1	Once a day	2	3 to 6 times a week	3	Once or twice a week	4	Not at all	5	
3. During the past **4 weeks**, how often did your **asthma** symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning?	4 or more nights a week	1	2 or 3 nights a week	2	Once a week	3	Once or twice	4	Not at all	5	
4. During the past **4 weeks**, how often have you used your rescue inhaler or nebulizer medication (such as albuterol)?	3 or more times per day	1	1 or 2 times per day	2	2 or 3 times per week	3	Once a week or less	4	Not at all	5	
5. How would you rate your **asthma** control during the **past 4 weeks**?	Not controlled at all	1	Poorly controlled	2	Somewhat controlled	3	Well controlled	4	Completely controlled	5	
											TOTAL

Copyright 2002, by QualityMetric Incorporated.
Asthma Control Test is a trademark of QualityMetric Incorporated.

If your score is 19 or less, your asthma may not be controlled as well as it could be. Talk to your doctor.

FOR PHYSICIANS:

The ACT is:

- A simple, 5-question tool that is self-administered by the patient
- Clinically validated by specialist assessment and spirometry[1]
- Recognized by the National Institutes of Health

Reference: 1. Nathan RA et al. *J Allergy Clin Immunol.* 2004;113:59-65.

2.5.2.3. Questionnaire of asthmatic children

Patient.name: Gender: Age:

Phone number:

Control treatment:

Montelukast. 4mg. 5mg. 10mg

Duration of therapy:

IgE level:

Pulmonary function test: FEV1.

2.6. Clinical biomarker

2.6.1. Total Serum Immunoglobin E level in asthmatic children

2.6.1.1. Principle of the assay

The antigen antibody interaction, in which an antibody selectively recognizes an antigen to create an immunological complex, provides the basis for the quantification of IgE. The antigen in these tests is serum IgE that reacts against the antibody in the serum sample. These assays involve the specific antibody for the Fc component of the IgE being adsorbed into a solid phase, often in polystyrene or cellulose wells. The purpose of this also, called "primary antibody" or "capture anti-body" is to bind to the IgE in the serum sample in order to create a complex with stable bound. In order to quantify this immunological complex, a second antibody known as a "secondary antibody "or "detection antibody" is used. This antibody is attached to an enzyme that enables the production of a colored substrates (ELISA) in an antigen dependent way. Simultaneously, a calibration curve with known concentrations of the analyte to be measured is processed to extrapolate the results of absorbance (in colorimetric methods, ELISA) to a protein concentration, which is then given in g, ng, or IU (Salazar, *et al.* 2017).

2.6.1.2. Procedure

The following procedure were carried out in accordance with the euroimmun kit:

1) Add 100 µL of serum samples into 900 µL sample buffer and thoroughly mix by vortex.

2) Place 100µL of diluted patient samples, positive controls, or calibrators into each microplate well. Then at room temperature (between 18 and 25 degrees), incubate the mixture for 30 minutes.

3) Apply 450 µL of wash buffer to reagent well and repeating the washing process three times (Automatic washing step).

4) In each well, the wash buffer should be left for 30 to 60 seconds during each washing cycle before being emptied.

5) In order to completely remove any liquid from the microplate after washing (both manual and automated tests), tap it on absorbent tissue paper with the opening facing downward. This will get rid of any remaining wash buffer.

6) Fill each microplate well with 100 µL of the enzyme conjugate (anti-human IgE that has been peroxidase labelled).

7) After that a 30 minutes of room temperature incubation is required.

8) Wash empty wells as previously mentioned.

9) Fill each well of the microplate with 100µL of the chromogen/substrate solution.

*Fifteen minutes of room temperature incubation with protection from the sun are required.

10) Transfer 100 of µL the stop solution into each microplate well in the same manner as pipette the chromogen/substrate solution: the same sequence and at the same speed.

11) Within thirty minutes of administering the stop solution, the color intensity should be measured photometrically with a wavelength near 450 nm as well as a wavelength of reference within 620 nm and 650 nm.

*Shake microplate just a little to ensure that the fluid is distributed evenly before measuring.

2.7. Molecular Analysis

2.7.1. Extraction of genomic DNA from blood sample

At the College of Pharmacy /University of Kerbala, DNA extraction was performed according to the instruction of frozen blood genomic DNA extraction kit (Macrogen, Taiwan).

The steps below demonstrate how to apply the principle of DNA extraction:

1. Before starting the procedure the frozen blood lifted at room temperature for few minutes. Then 200 µL of blood were add to 1.5 ml epindorff tube.
2. Add 30 µL of resuspended proteinase K (10mg/1.1ml) and then mixed briefly and incubating for 15 minutes at 60 temperature in incubator.
3. Add 200 µl of FABG buffer to the sample. By pulsing and vertexing, completely combine.
4. To lyse the sample, place it in an incubator at 70 °C for 15 minutes, and shake the sample every three to five minutes during incubation.
5. Elution Buffer placed in an incubator at 70 °C to preheat the buffer.
6. Add 200 µl of absolute ethanol to the sample. For 30 seconds, vortex in pulses to thoroughly mix. Quickly spin the tube to clear any liquid from the lid's interior.
7. Insert an FABG column to a collection tube. Carefully transfer the sample mixture together with any precipitate to the FABG Column. Centrifuge for one minute at speed 14000rpm (18000xg), then remove the flowthrough and insert fresh FABG column.
8. Immediately run 400 µl of W1 Buffer through the FABG Column and discard the flow-through.
9. Centrifuge the FABG Column for 1 minute while washing it with 600 µl of Wash Buffer, then throw away the flow-through. When opening, check to see if ethanol has been added to the wash buffer.

10. To dry the column, centrifuge for an additional 3 minutes. By doing this action, the remaining liquid won't be able to stop further enzymatic reactions.
11. Insert the FABG Column into the new 1.5 ml epindorff tube. Fill the membrane center of FABG column with 100 µl of elusion buffer and centrifuge for 1 minute.
12. Store the DNA fragment at 4^{o}C or -20^{o}C.

2.7.2. Allele Specific Polymerase Chain Reaction

Allele-specific (AS- PCR) was used for detection of single nucleotide polymorphisms (SNPs) of CYP2C8*1B (rs7909236) and OATP2B1 c.935G>A (rs12422149) as prescribed by Darawi *et al* (2013).

2.7.3. Primers

The primers were designed by Professor Dr. Hassan Mahmood, using Primer Blast software (http: www.ncbi.nih.gov/tools/primer-blast) and send to Macrogen compony for further production. The received lipolyzed primers were diluted and reconstituted in the following ways: Before being removed from the centrifuge, the tube was spun at 10000 x g for 5-10 minutes.

Lyophilized primers then dissolved in a nuclease-free water to create a (stock solution) by adding the desired volume in accordance with the manufacturer's instructions to produce an initial concentration of 100 pmoles/L. For the working solution, the primers were mixed once more using an appropriate vortex, and then 10Pmol of the stock solution were diluted with 90 Pmol of nuclease free water in a 0.5 ml eppendrof tube to obtain (10pmol) as a working solution, the stock and working solution were stored at -20 ^{o}C (Ye, *et al* ,2012).

The forward and reverse sequences of primers and product sizes are listed in tables (2-3) and (2-4).

Table (2-3) Primer Sequence of OATP2B1 transporter gene c.935G>A

Alleles	Sequence (5'->3')
Allele G	GTGAGCTTCAGTTTCGGCG
Allele A	GTGAGCTTCAGTTTCGGCA
Reverse primer	AGGCAGAAAGTATAGTGCCCA
Products length 467	

Table (2-4) Primer Sequence of CYP2C8*1B enzyme gene g. -271 c> A

Alleles	Sequence (5'->3')
Forward primer	CAGCACCAGGACCACAAAAG
Allele G	ATCATCACAGCACATTGGAAC
Allele T	ATCATCACAGCACATTGGAAA
Product length 318	

2.7.4. Polymerase Chain Reaction (PCR) working solution

In each PCR tube of the Accupower ® PCR Pre Mix Bioneer Kit there is a lyophilized mixture of the following ingredients: dNTPs, Tris-HCl, KCl, Enhancer (MgCl2), Stabilizer, and Tracking Dye. This kit is ready to be used and for preparing PCR working solution, only add the DNA template and primers as prescribed in kit procedure.

2.7.5. Optimization of Polymerase Chain Reaction (PCR) Conditions

Optimization of PCR was accomplished after several trials. PCR was prepared using different volumes of primer and template DNA with different annealing temperature.

The desired conditions for OATP2B1 c.935G>A can be achieved using:

A. 6 µL of DNA sample

B. 0.5 µL of forward primer

C. 0.5 µL of reverse primer

D. 13 µL of nuclease free water

20 µL total reaction volume is added to the 5 µL PCR tube of premix, which contains 5 µL of premix at 25°C. The sample tubes are then mixed in the micro centrifuge for 10 seconds at 2000 xg before being placed in the thermocycler. The following program in table (2-5) represents the best PCR reaction.

Table (2–5) Lists of the requirements for OATP2B1 transporter gene c.935G>A optimization

Steps	Temperature	Time	Cycles
Initial Denaturation	95	5 min	1
Denaturation	95	20 sec	35
Annealing	57.8	20 sec	35
Extension	72	40 sec	40
Final extension	72	5 min	1

The desired conditions for CYP2C8*1B (rs7909236) can be achieved using:

A. 6 µL of DNA sample

B. 1 µL of sense primer

C. 1 µL of antisense primer

D. 12 µL of nuclease free water

Table (2–6) Conditions for CYP2C8*1B enzyme gene optimization.

Steps	Temperature	Time	Cycles
Initial Denaturation	95	5 min	1
Denaturation	95	20 sec	35
Annealing	54.1	20 sec	35
Extension	72	35 sec	40
Final extension	72	5 min	1

2.8. Agarose gel electrophoresis

Throughout the agarose gels, DNA fragments with varying sizes ranging from 100 bp to 1000 bp are identified, separated, and purified using electrophoresis as a common method. Agarose gel electrophoresis was used to verify PCR amplification (Ylmaz,*et al*,.2012).

2.8.1. Preparation of 1x Tris Borate EDTA (TBE) solution

One volume of 10X TBE buffer was diluted with nine volumes of deionized water, yielding a 1:10 dilution to produce 1X TBE buffer solution (tris borate EDTA).

2.8.2. Preparation of agarose gel

1.5 g of agarose powder should be dissolved in 100 ml of 1x TBE buffer, then the solution should be heated until all particles are dissolved and the gel solution becomes clear and pure. The temperature of the solution was lowered to between 50 and 60 °C. Then 1.5 ml of stock ethidium bromide was added (Ylmaz, *et al.* 2012).

2.8.3. Casting of the horizontal agarose gel

The comb was inserted about 1 inch to the end of the tray in gel chamber to create wells, then the agarose gel was poured into the tray and left to solidify at room temperature for approximately 30 minutes before being refrigerated for 15 to 30 minutes to ensure proper

hardening. The comb was removed carefully from the tray and fixed in horizontal electrophoresis tank filled with 1X TBE buffer.

2.8.4. Loading of Polymerase Chain Reaction (PCR) products

After the gel hardened the comb was removed carefully from the tray and the glass plates were put in submarine horizontal electrophoresis tank filled with 1X TBE buffer. The wells were then directly loaded with 5 µL of PCR products. The positive pole was connected to positive side of unit, and the negative pole to another. Electrical power was then turned on at 100 volt/50 Amp for 60 minutes or while waiting for dye indicators traveled to the proper distance corresponding to the size of DNA fragments. From the cathode, DNA migrates to the anode. A gel Documentation System was used to view the bands stained with Ethidium Bromide.

2.8.5. Molecular Weight Marker

In this study, 3 µL of DNA ladder and 3 µL of loading dye was used (SolGent, Korea) and the band size ladder was 100-3000 bp.

2.8.6. Gel- Band Visualization

The agarose gel was put in a UV transilluminator device in order to expose it to UV light in an attempt to detect DNA bands, and pictures were captured with a digital camera.

2.9. Statistical analysis

The obtained results were expressed as mean ± standard deviation (SD) and by percent using the Statistical Program for the Social Sciences (SPSS) version 24.

The analysis of variance (ANOVA) was used to assess differences between groups, whereas one sample t-test was used to analyze the total IgE level. Differences in data reported by percent (less than 5) in genotype groups were analyzed using Fisher's exact test and Chai square test was used to evaluate the goodness of fit between Asthma Control Test (ACT) score. Pearson's Correlation was utilized to assess the relationship between clinical parameters and genotype groups in asthmatic children and Hardy-Weinberg equilibrium was used to calculate the genetic variation of a population at equilibrium. The significance level (P-value) for all the statistical tests was set at less than 0.05.

Chapter Three

Results

3. Results

3.1. Demographic characteristic of asthmatic children

The age of the included patients (N=100) ranged from 6 to 15 years with a mean of 9.55 ±2.66 years. The male to female ratio was 1.7:1. This information is particularly important in pulmonary function test to predict the pulmonary function for each child according to the age, height, weight, BMI and gender as shown in table (3.1).

Table (3-1): Demographic characteristics of asthmatic children.

Asthmatic Children (N=100)		
Data Presented by Mean ± SD		
Age (years)		9.55± 2.67
Duration of treatment(months)		3.57 ± 1.53
Weight (kg)		33.8± 14.49
Height (cm)		135 ± 13.08
Data Presented by Percent %		
Age group (years)	Ages 6-9	46 (46%)
	Ages 10-15	54 (54%)
BMI (kg\m²)	Underweight (<5th percentile)	16 (16%)
	Healthy weight (5th - 85th percentile)	62 (62%)
	Overweight (85th -less than 95th percentile)	5 (5%)
	Obesity (95th ≥ percentile)	17 (17%)
Gender N (%)	**Male**	64 (64%)
	Female	36 (36%)

BMI: Body mass index, N: Numbers of the Study participant, SD: Standard deviation.

3.2. Asthma control tools for asthmatic children

Asthma control tools which were demonstrated by control of asthma symptoms (using asthma control test) and pulmonary function (using FEV1 and PEF parameters).

Asthma Control Test (ACT) showed that (21) of asthmatic children responded well to montelukast therapy (score ≥20), while (79) of asthmatic children responded poorly to montelukast therapy (score≤19) and the difference between these two groups was highly significant (P value <0.05) as shown in table (3-2).

Table (3-2): Asthma Control Test (ACT) for asthmatic children

Asthma Control Tools in Children (100)			P value
Data Presented by Percent N (%)			
Asthma Control Test Score	Controlled asthma (≥20)	21(21%)	**0.001***
	Uncontrolled asthma (≤19)	79(79%)	

*Chi square test was used with a significance p value of less than 0.05.

The result of pulmonary function test show that the mean of FEV1 and PEF was not significantly different (p value <0.05) in both controlled and uncontrolled group when compared with normal value that was obtained from previous study of Chipps (2008) as shown in table (3-3).

Table (3-3): Pulmonary Function Test (PFT) among controlled and uncontrolled groups of asthmatic children

Pulmonary Function Parameters		Mean ± SD	Normal value	P value
FEV1 (L)	Controlled asthma (n=21)	83.76±13.37	≥80%	0.21
	Uncontrolled asthma(n=79)	81.6±10.77		0.18
PEF (L/S)	Controlled asthma (n=21)	82.47±11.92	≥80%	0.35
	Uncontrolled asthma(n=79)	79.17±11.93		0.54

FEV1: Force Expiratory Volume in 1 second PEF: Peak Expiratory Force.

*One sample t-test was used with a significant p value of less than 0.05.

3.3 Serum Total Immunoglobulin E level for asthmatic children

This study showed that the serum total IgE level in the uncontrolled asthma of both group 1 and 2, shows highly significant (p value less than 0.05) difference when it was compared with the normal value obtained from kit leaflet as mentioned in table (3-4).

Table (3-4): Serum Total Ig E levels among controlled and uncontrolled groups of asthmatic children.

Patients Groups		IgE (IU/ml) Mean ± SD	Normal value (IU/ml)	P value
Group 1 (Age 6-9) n= 46	**Controlled group (n=14)**	172.35±46.69	155	0.18
	Uncontrolled group (n=32)	182.2±33.13		**0.001***
Group 2 (Age 10-15) n=54	**Controlled group (n=7)**	188.85±21.72	199	0.26
	Uncontrolled group (n=47)	218.93±47.66		**0.006***

Results expressed as mean ±SD

*One sample t-test was used with a significant P value of less than 0.05.

3.4. Genetic Amplification Reactions (results of genotypes analysis)

The genetic amplification reaction was Allele specific PCR that was used for both genes and the results are shown in each following separated topic.

3.4.1. Genotype of OATP2B1 c.935G>A transporter gene polymorphism of asthmatic children

The results of OATP2B1 c.935 G>A (rs12422149) transporter gene polymorphism was a single clear band with a molecular size 467 bp (figure 3-1). The size of amplicon was determined by compare with DNA ladder 100 - 3000 bp. Genetic polymorphism of this gene were classified into three genotypes; one homozygous for the G allele (GG) wild type, one heterozygous (GA) and the last one was homozygous for the allele A (AA) mutant type. The following figure (3-1) and table (3-5) illustrate the gel electrophoresis and frequency of these alleles in the all participants and in controlled and uncontrolled asthma groups.

Table (3-5): Distribution of different genotype of OATP2B1 c.935G>A transporter gene polymorphism of asthmatic children

Patient Genotype	N (%)	Controlled Asthma Group (n=21)	Uncontrolled Asthma Group (n=79)
Wild Homozygous (GG)	48 (48%)	17 (17%)	31 (31%)
Mutant Heterozygous (GA)	45 (45%)	4 (4%)	41 (41%)
Mutant Homozygous (AA)	7 (7%)	0	7 (7%)
Total	**N= 100 (100%)**		

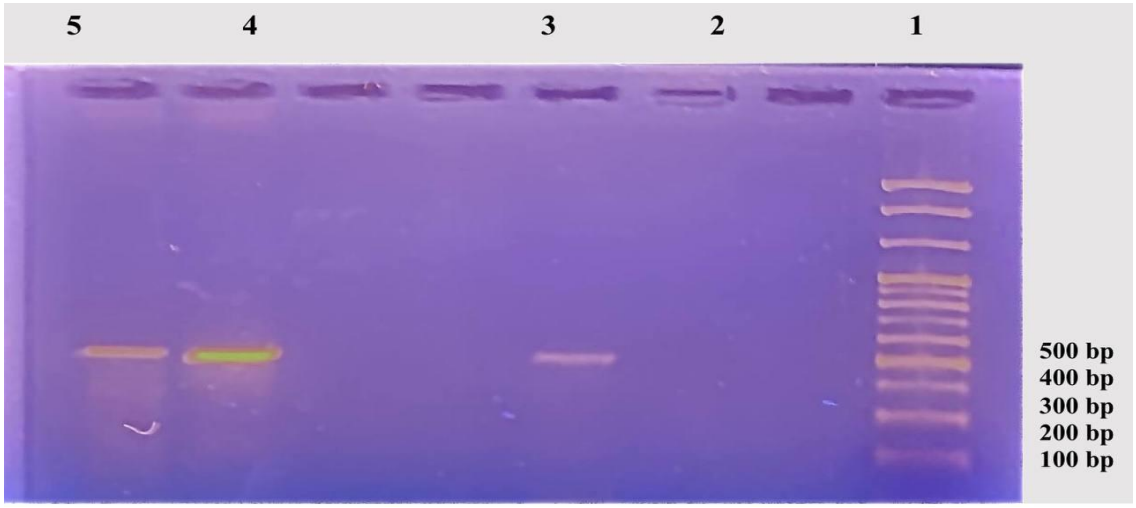


Figure (3-1) Electrophoresis of agarose gel for allele specific PCR product of OATP2B1 transporter gene c.935G>A polymorphisms showed: Lane 1: Represented DNA ladder 100-3000 bp, Lane (2,3): Represented (one sample) mutant homozygous AA genotype, Lane (4, 5) represented (one sample) mutant heterozygous GA genotype.

The size of amplicon for OATP2B1 c.935G>A (rs12422149) transporter gene polymorphism was 467 bp that measured by comparison with DNA ladder band (100-3000bp).

3.4.1.1. Hardy-Weinberg Equilibrium for OATP2B1 c.935G>A transporter gene polymorphism in asthmatic children

The Hardy-Weinberg Equilibrium for OATP2B1 c.935G>A transporter gene polymorphism in observed asthmatic children compared with expected frequency of equilibrium and the result show that there were no significant differences (p value <0.05) between observed and expected ones. This result states that the amount of genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors.

Table (3-6): Hardy-Weinberg Equilibrium for OATP2B1 c.935G>A transporter gene polymorphism of asthmatic children

Genotypes	GG	GA	AA	P value
Observed	48 (48%)	45 (45%)	7(7%)	0.41
Expected	49.7(49.7%)	41.59(41.59%)	8.7(8.7%)	

* Chi square test was used with a significant p value of less than 0.05.

3.4.2. Genotyping of CYP2C8*1B enzyme gene g.-271C>A polymorphism of asthmatic children

The agarose gel electrophoresis and distribution of different genotype of CYP2C8*1B enzyme are represented in following figure (3-2) and table (3-7), respectively.

The results of CYP2C8*1B enzyme gene g.-271C>A (rs7909236) polymorphism was a single clear band with a molecular size 318 bps. (Figure 3-2) The size of amplicon was determined by compare with DNA ladder 100 - 3000 bp, Genetic polymorphism of this gene were classified into three genotypes; one homozygous for the A allele (CC) wild type, one heterozygous (CA) and the last one was homozygous for the allele A (AA) mutant type.

Table (3-7): Distribution of different genotype of CYP2C8*1B enzyme gene g.-271C>A polymorphism of asthmatic children

Patient Genotype	N (%)	Controlled Asthma Group (n=21)	Uncontrolled Asthma Group (n=79)
Wild Homozygous (CC)	74 (74%)	16 (16%)	**58 (58%)**
Mutant Heterozygous (CA)	22 (22%)	5 (5%)	**17 (17%)**
Wild Homozygous (AA)	4 (4%)	0	**4 (4%)**
Total	**N=100 (100%)**		

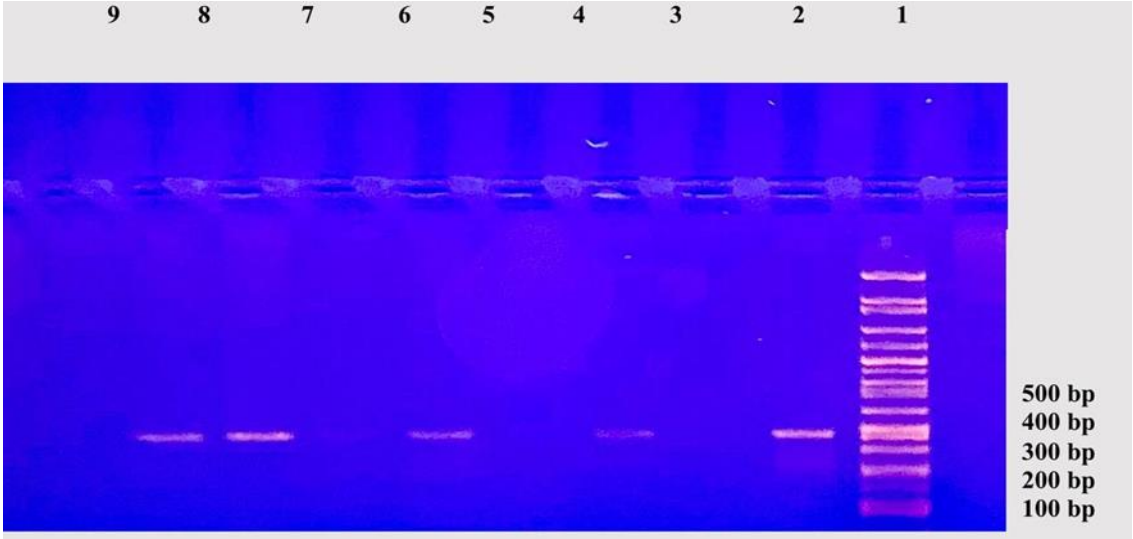


Figure (3-2): Electrophoresis of agarose gel for allele specific PCR product of CYP2C8*1B enzyme gene g.-271C>A polymorphism showed: Lane 1: Represented DNA ladder 100-3000 bp, Lane (2-7): Represented (3 sample) wild homozygous CC genotype, Lane (8,9) represented (one sample) mutant heterozygous CA genotype.

3.4.2.1. Hardy-Weinberg Equilibrium for CYP2C8*1B g.-271 C> A enzyme gene polymorphism in asthmatic children

The Hardy-Weinberg Equilibrium for CYP2C8*1B enzyme gene g.-271 C>A polymorphism in observed asthmatic children compared with expected frequency of equilibrium and the result show that there were no significant differences (p value <0.05) between observed and expected ones. This result states that the amount of genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors.

Table (3-8): Hardy-Weinberg Equilibrium for CYP2C8*1B enzyme gene g.-271 C>A polymorphism in asthmatic children

Genotypes	CC	CA	AA	P value
Observed	74 (74%)	22 (22%)	4 (4%)	0.16
Expected	72.25 (72.25%)	25.5 (25.5%)	2.25 (2.25%)	

* Chi square test was used with a significant p value of less than 0.05.

3.5. Demographic characteristics of different genotypes of OATP2B1 c.935G>A transporter gene and CYP2C8*1B g.-271C>A enzyme gene polymorphisms in asthmatic children

The results demonstrated that there was no significant difference ($p > 0.05$) between the demographic of the asthmatic children and their corresponding genotype as mentioned in tables (3-9, 3-10, 3-11, 3-12).

Table (3-9) Demographic characteristic of different genotypes of OATP2B1 c.935G>A transporter gene polymorphisms in asthmatic children

Demographic parameters	Patients Genotypes N=100			P value
	GG (n=48)	GA (n=45)	AA (n=7)	
Age (years)	9.56 ±2.44	9.51±2.76	9.71±3.94	0.85
Weight (kg)	34.35±13.84	33.17 ±15.97	34±9.36	0.69
Height (cm)	136.73±12.9	133.69±13.3	133.14±13.4	0.26
Duration of therapy (Months)	3.97±1.69	3.24±1.33	2.85±0.89	0.053

Results were express as mean ± SD

*ANOVA test was used with a significant p value of less than 0.05.

Table (3-10) Association between demographic characteristic and different genotypes of OATP2B1 c.935G>A transporter gene polymorphisms in asthmatic children

Demographic parameters		Patients' genotypes (N=100)			P value
		GG	GA	AA	
BMI (kg/m2)	Under weight	7(7%)	8(8%)	1(1%)	0.063
	Healthy wight	32(32%)	26(26%)	4(4%)	
	Over weight	1(1%)	4(4%)	0	
	Obesity	8(8%)	7(7%)	2(2%)	
Gender	Male	34(34%)	27(27%)	3(3%)	0.24
	Female	14(14%)	18(18%)	4(4%)	

Results were express as percent.

*Fisher's Exact test was used with a significant p value of less than 0.05.

Table (3-11) Demographic characteristics of different genotypes of CYP2C8*1B g.-271C>A enzyme gene polymorphism in asthmatic children

Demographic parameters	Patients Genotypes (N=100)			P Value
	CC (n=74)	CA (n=22)	AA (n=4)	
Age (years)	9.68±2.74	9.22±2.36	8.75±3.4	0.48
Weight (kg)	34.12±14.21	34.45±16.22	24.25±6.65	0.18
Height (cm)	135.64±12.88	135.18±13.71	124.75 ±11.87	0.10
Duration of therapy (Months)	3.48±1.51	3.9±1.68	3.25±0.95	0.26

Results were express as mean ± SD

*ANOVA test was used with a significant p value of less than 0.05.

Table (3-12) Association between demographic characteristics and different genotypes of CYP2C8*1B g.-271C>A enzyme gene polymorphism in asthmatic children

Demographic parameters		Patients' genotypes (N=100)			P value
		CC	CA	AA	
BMI (kg/m^2)	Under weight	12 (12%)	3(3%)	1 (1%)	0.62
	Healthy weight	44 (44%)	15 (15%)	3(3%)	
	Overweight	5 (5%)	0	0	
	Obesity	13 (13%)	4 (4%)	0	
Gender	Male (n=64)	43(43%)	18(18%)	3(3%)	0.083
	Female (n=36)	31 (31%)	4(4%)	1(1%)	

Results express as percent.

*Fisher' Exact was used with significant p value of less than 0.05.

3.6. Effects of different genotypes of OATP2B1 c.935G>A transporter gene polymorphism on montelukast therapy response in asthmatic children

This study showed that approximately 21 patients (17 with GG and 4 with GA genotypes) with OATP2B1 c.935G>A transporter gene polymorphism responded well to montelukast therapy that manifested by controlled asthma symptoms (≥20 score in Asthma Control Test (ACT)), significant reduction (P value < 0.05) of IgE level, accompanied by a marked improvement in pulmonary function in comparison with GA genotype as shown in the tables (3-13, 3-14, 3-15,3-16).

Regarding uncontrolled asthma group, about 79 patients (31 with CC, 41 with CA, and 7 with AA genotypes) responded poorly to montelukast therapy demonstrated by uncontrolled asthma (≤19 score in Asthma Control Test (ACT)). In this group (uncontrolled asthma group) GG group show better response (more reduction on IgE level and better improvement on

FEV1 and PEF) when compared with GA and AA genotypes as shown in the tables (3-13, 3-14, 3-15, 3-16).

Table (3-13): Association between Asthma Control Test (ACT) and different genotypes of OATP2B1 c.935G>A transporter gene polymorphism in asthmatic children

Asthma controlled Test (ACT) Score	Patients Genotypes N=100			P value
	GG	GA	AA	
Controlled asthma (≥20)	17(17%)	4(4%)	0	0.002*
Uncontrolled asthma (≤19)	31(31%)	41(41%)	7(7%)	

*Fisher's Exact was used with a significant p value of less than 0.05.

Table (3-14): Mean of Serum Total IgE Levels of group 1 among different genotypes of OATP2B1 c.935G>A transport gene polymorphism in controlled and uncontrolled groups of asthmatic children

Patient Group 1 Ages (6-9) N=46	Patients Genotype of Controlled Group			P value
	GG n=11	GA** n=3	AA n=0	
Controlled asthma N= 14	149.45±6.03	256.33±23.02	0	0.014*
Uncontrolled asthma N=32	Patients Genotypes of Uncontrolled Group			P value
	GG n= 11	GA** n=18	AA n=3	
	154.18±16.98	196.50±32.02	198.33±17.55	0.001*

Results were express as mean ± SD.

*T- test was used for controlled and ANOVA test was used for uncontrolled asthma with a significant p value of less than 0.05.

** There was significant difference between GG and GA group.

Table (3-15): Mean of Serum Total IgE Levels of group 2 among different genotypes of OATP2B1 c.935G>A transport gene polymorphism in controlled and uncontrolled asthmatic children

Patient Group 2 Ages (10-15) N=54	Patients Genotype of Controlled Group			P value
	GG n=6	GA** n=1	AA n=0	
Controlled asthma N=7	182±13.09	230	0	**0.019***
Uncontrolled asthma N=47	Patients Genotypes of Uncontrolled Group			P value
	GG n=20	GA** n=23	AA n=4	
	193.25±29.88	236.43±51.64	246.75±42.90	**0.005***

Results were express as mean ± SD.

*T-test was used for controlled and ANOVA test was used for uncontrolled asthma with a significant p value of less than 0.05.

** There was significant difference between GG and GA group.

Table (3-16): Pulmonary Function parameters of controlled and uncontrolled asthma groups among different genotypes of OATP2B1 c.935G>A transporter gene polymorphism in controlled and uncontrolled group of asthmatic children

Pulmonary function Parameters	Asthma Groups	Patients Genotypes N=100			P value
		GG n=17	GA n=4	AA n=0	
FEV1 (L)	Controlled asthma N=21	81.76±14	92.25±5.31	0	0.16
	Uncontrolled asthma N=79	GG n=31	GA n=41	AA n=7	P value
		83.87±10.53	81.63±10.50	71.42±8.46	**0.005***
PEF (L/S)	Controlled asthma N=21	GG n=17	GA n=4	AA n=0	P value
		80.76±12.21	89.75±8.09	0	0.18
	Uncontrolled asthma N=79	GG n=31	GA n=41	AA n=7	P value
		83.16±9.61	77.75±13.04	69.85±7.64	**0.007***

Results were express as mean ± SD.

*T-test was used in controlled asthma and ANOVA test was used in uncontrolled asthma groups with a significant p value of less than 0.05.

**There was highly significant difference between GG and AA genotypes.

Table (3-17) illustrate that the patient of current study, there was correlation between OATP2B1 transporter gene c.935G>A polymorphism and serum total IgE levels in both groups (1 and 2), and PEF (the p value

was significant). But there was no correlation between OATP2B1 transporter gene c.935G>A polymorphism and FEV1.

Table (3-17) Pearson's correlation between OATP2B1 c.935G>A transporter gene polymorphism and clinical parameters in asthmatic children

Clinical parameters	Pearson's correlation	P value
Serum total IgE in group 1	0.63	**0.01***
Serum total IgE in group 1	0.49	**0.01***
FEV1	-0.18	0.07
PEF	-0.25	**0.01***

* Pearson's correlation was used with a significant p value of less than 0.05.

3.7. Effects of different genotypes of CYP2C8*1B g.-271C>A enzyme gene polymorphism on montelukast therapy response in asthmatic children

This study showed that approximately 21 patients (16 with CC and 5 with CA genotypes) with the CYP2C8*1B enzyme polymorphism responded well to montelukast therapy manifested by an controlled asthma symptom (≥20 score of Asthma Control Test (ACT)), but with non-significant differences in IgE level, accompanied by pulmonary function parameter (FEV1 and PEF) among wild (CC) and mutant (CA) genotype, as shown in the tables (3-18, 3-19, 3-20,3-21).

Regarding uncontrolled asthma group, about 79 patients (58 with CC, 17 with CA, and 4 with AA genotypes) responded poorly to montelukast therapy demonstrated by uncontrolled asthma symptoms (≤19 score of Asthma Control Test (ACT)), a non-significant difference in IgE level, but a significant difference on pulmonary function parameter (FEV1 and PEF)

among wild (CC) and mutant (CA and AA) genotype, as shown in the tables (3-18, 3-19, 3-20, 3-21).

Table (3-18): Association between Asthma Control Test and different genotypes of CYP2C8*1B g.-271C> A enzyme gene polymorphism in asthmatic children

Asthma Control Test (ACT) Score	Patient Genotype (N=100)			P Value
	CC	CA	AA	
Controlled asthma (≥20)	16(16%)	5(5%)	0	0.81
Uncontrolled asthma (≤19)	58(58%)	17(17%)	4(4%)	

Results were express as percent.

*Fisher's Exact was used with a significant p value of less than 0.05.

Table (3-19): Serum Total IgE Levels of group 1 of controlled and uncontrolled asthma groups among different genotypes of CYP2C8*1B g.-271C>A enzymes gene polymorphism in asthmatic children

Patient Group 1 Ages (6-9) N=46	Patients Genotype of Controlled Group			P value
	CC n=11	CA n=3	AA n=0	
Controlled asthma N= 14	162.18±39.41	209.66±61.24	0	0.12
Uncontrolled asthma N=32	Patients Genotypes of Uncontrolled Group			P value
	CC n= 22	CA n=8	AA n=2	
	183.22±35.17	181.5±32.14	172.5±24.74	0.9

Results express as mean ± SD

*T-test was used for controlled asthma and ANOVA test was used for uncontrolled group with a significant p value of less than 0.05.

Table (3-20): Serum Total IgE Levels of group 2 of controlled and uncontrolled groups among different genotypes of CYP2C8*1B g.-271C>A enzymes gene polymorphism in asthmatic children

Patient Group 2 Ages (10-15) N=54	Patients Genotype of Controlled Group			P value
	CC n=5	CA n=2	AA n=0	
Controlled asthma n= 7	192.2±21.15	173±18.38	0	0.25
Uncontrolled asthma n=47	Patients Genotypes of Uncontrolled Group			P value
	CC n=36	CA n=9	AA n=2	
	220.61±47.31	213.11±56.19	215±21.21	0.68

Results express as mean ± SD

*T-test was used for controlled asthma and ANOVA test was used for uncontrolled group with a significant p value of less than 0.05.

Table (3-21): Pulmonary Function parameters of controlled and uncontrolled group among different genotypes of CYP2C8*1B g.-271C>A enzymes gene polymorphism in asthmatic children

Pulmonary function Parameter	Asthma Groups	Patients Genotypes N=100			P value
		CC n=16	CA n=5	AA n=0	
FEV1 (L)	Controlled asthma N=21	82±14.95	89.4±1.94	0	0.071

	Uncontrolled asthma N=79	**CC n=58**	**CA** n=17**	**AA n=4**	**P value**
		83.18±9.84	75.11±12.22	86.25±7.93	**0.006***
PEF (L/S)	**Controlled asthma N=21**	**CC n=16**	**CA n=5**	**AA n=0**	**P value**
		80.62±12.87	88.4±5.68	0	0.076
	Uncontrolled asthma N=79	**CC n=58**	**CA** n=17**	**AA n=4**	**P value**
		80.82±11.52	72.41±12.27	84±5.77	**0.01***

Results were express as mean± SD

*T-test was used in controlled and ANOVA test was used in uncontrolled group with a significant p value of less than 0.05.

** There was significant difference between CC and CA group.

The table (3-22) the Pearson's correlation was used between clinical parameters and different genotypes groups of CYP2C8 g.-271C>A enzymes genes polymorphism. The result show no significant corelation between clinical parameters and different genotypes groups of CYP2C8 g.-271C>A enzymes genes polymorphism in asthmatic children.

Table (3-22) Pearson's correlation between CYP2C8 g.-271 C>A enzyme gene polymorphism and clinical parameters in asthmatic patients

Clinical parameters	Correlation coefficient	P value
Serum total IgE level in group 1	0.089	0.55
Serum total IgE level in group 1	-0.078	0.57
FEV1	-0.48	0.63
PEF	-0.4	0.69

*Pearson Correlation coefficient was used with a significant p value of less than 0.05.

Chapter Four

Discussion

4. Discussion

There is evidence suggesting that individuals from various cultures and ethnic groups react to asthma treatments differently. This is probably due to genetics variations inherited from a particular origin connected with the severity of the condition or how well a person responds to treatment. (Ferrante *et al.,*2022).

This cross-sectional observational study done on asthmatic children, demonstrate the rationale behind conducting asthma pharmacogenetics studies, provide an overview and baseline of OATP2B1 transporter and CYP2C8*1B enzyme gene polymorphisms, and detail the most important results of OATP2B1 transporter gene c.935G>A and CYP2C8*1B enzyme gene g.-271C>A polymorphisms on Montelukast therapy response in sample of Iraqi asthmatic children.

4.1. Demographic features of asthmatic children

The demographic data was used to estimate the prevalence of asthma in participants based on their age and gender.

The age of patients in this study ranged from 6 to 15 years with a mean ± SD of 9.55 ±2.67, and those younger than 6 were excluded because performing PFT and ACT on them was difficult. According to the BMI classification, 62% of participants were in healthy weight. These findings are consistent with a study conducted in Sudia Arabia showed that 28.13% of the participants were overweight or obese and 71.88% of them were normal to underweight (Alolyan *et al*.,2021).

Depending on the gender of participants, this study showed that about 64(64%) of males and 36(36%) of females suffer from asthma, which is agreed with previous studies found that male is more susceptible to asthma than female (Al Thamiri *et al*.,2005; Yao *et al.*, 2011).

4.2. Asthma control tools in asthmatic children

In this study, the Asthma Control Test (ACT) and Pulmonary function Test (PFT) were used to described asthma control in children with mild to moderate asthma (Global Initiative for Asthma (GINA, 2023).

According to ACT, participant patients were considered to have managed asthma if their ACT score remined greater than 20. Only 21(21%) of participants had controlled asthma (≥20), and 79(79%) had uncontrolled asthma (≤19). These results were in consistent with previous study carried out on 879 children with asthma aged 12 to 16 years in the Greater Toronto Area, in which only 11% of them had controlled asthma (Ungar *et al*., 2015). On the other hand, this result was in contrast with another study conducted in Riyadh, which found that 67.19% of participating asthmatic children had controlled asthma (Alolayan *et al*,2021). This difference in results might be attributed to variations in the environment, sample size, and the assessment tools (Banjari *et al*.,2018).

A mix of clinical and functional indicators, occasionally in conjunction with inflammatory disease markers, can be used to evaluate the effectiveness of anti-asthma medication. Peak expiratory flow (PEF) and forced expiratory volume in one second (FEV1) are two functional end goals that are frequently employed in clinical trials. The pathophysiology in the small airways may not be sufficiently addressed by these end points because they primarily concentrated on the bigger airways (Pesant *et al.,* 2007). In step 2 of the asthma severity scale, individuals with mild persistent asthma are advised to take an anti-inflammatory medication daily as a controller therapy (Expert Panel Report 3, Asthma Guideline ,2020). Over 30% of asthma patients fall into this category, which is classified as having near-normal lung function but active asthma symptoms. In either adults or children, lung function does not significantly

correlate with asthma symptoms. Lung function is sometimes statistically averaged or coupled with symptoms in asthma control tools, but if the instrument has multiple symptom categories, these may overwhelm clinically significant variations in lung function. Even after accounting for symptoms frequency, low FEV1 remains a potent independent indicator of risk of exacerbations (GINA.2023).

4.3. Serum Total Immunoglobulin E level in asthmatic children

A biomarker for assessing treatment response is the total serum Ig E level. Numerous studies have shown that total Ig E levels correlate with asthma severity in children as well as adults' ancestry that serum Ig E levels increased as asthma severity increased (Maneechotesuwan, *et al.*, (2010).

In present study, total serum Ig E level was measured after at least 4 weeks of montelukast therapy and the results show that in both patient's groups (group 1 that ages between 6-9 and group 2 that ages between 10-15) the levels of total Ig E remain high in controlled and uncontrolled asthma groups despite montelukast treatment (table 3-4). This result is in consistent with a study that done by Hameed, *et al.* (2019), which found that children with moderate asthma had total serum IgE levels of 207.5 IU/ml, whereas those with mild asthma had levels of 164.3 IU/ml. It is also in consistent with the study of Kovac, which found that the IgE levels of asthmatic patients were 288.0 IU/L (Kovac, *et al*.2017). These observations are supported by the results of Sandeep, who discovered that Ig E levels increased as asthma severity increased (Sandeep, et. *al.*, 2010). Davila, in contrast, did not differ between IgE levels in mild and moderate asthma but exhibited an increase in total serum IgE (Davila, *et al* ,2015).

4.4. Genetics Amplification Reactions

Allele specific Polymerase Chain Reaction (PCR), used in genetic investigations, and the outcomes of this amplification reaction are explained in depth in the distinct sections that follow.

4.4.1. Distribution of OATP2B1 c.935G>A transporter gene polymorphism in asthmatic children

The distribution of different genotypes of OATP2B1 c.935G>A transporter gene polymorphism in asthmatic children of the current study was as follow: 48 (48%) for GG wild homozygous, 45 (45%) for GA mutant heterozygous, and 7 (7%) for AA mutant homozygous genotypes.

This outcome was comparable to that of Li, *et al.* (2019), who study on a sample of 50 Chinese individuals and found that the frequency of the allele of OATP2B1 gene c.935G>A polymorphism in this community reached; 42% for GG, 48% for GA, and 10% for AA genotypes. In contrast to another study (Kim, *et al.*,2013) of 227 healthy, Korean female and male found that the allele frequency was ;31% GG, 51% GA, and 17% AA, which is also was comparable to the findings of the present studies in Iraqi population.

The prevalence of these alleles was varied in Finnish-Caucasian of 552 healthy people; 86.4% for GG, 13.6% for GA, and AA genotypes not observed (Laitinen, *al et*.,2011). The difference in results could be attributed to variations in size of sample and sample subjects' ethnicity.

4.4.2. Distribution of CYP2C8*1B g.-271C>A enzyme gene polymorphism in asthmatic children

About 23% of white individuals and 10% of Asian people, but not Africans, have the CYP2C8*1B polymorphism, commonly referred to as the CYP2C8*1B gene g.-271 C>A polymorphism (Bahadur, *et al*,2002; Rodriguez, *et al* ,2008).

Allele's distribution in the current study was as follows:74(74%) for wild homozygous CC genotypes, 22 (22%) for mutant heterozygous CA genotypes, and 4 (4%) for mutant homozygous CC genotypes. In a conflict with the findings of Rodriguez-Antona, *et al. (*2008) with allele frequency of 24 % for CA genotypes. And in keeping with research conducted in Chinese patients, which predicted that the distribution of this allele was 92% for CC, 8% for CA, and AA genotypes not founded (Li, *et al.*, 2019).

4.5. Demographic characteristics of different genotypes of OATP2B1 c.935G>A and CYP2C8*1B g.-271C>A in asthmatic children

There were no significant differences (>0.05) between genotype groups of the genes OATP2B1 c.935G>A and CYP2C8*1B g.-271C>A, when it came to age, weight, BMI, or gender and may be all genotype groups have nearly equal patient capacity for performing the pulmonary function test.

4.6. Asthma Control Test (ACT) prevalence among different genotypes of OATP2B1 c.935G>A transporter gene and CYP2C8*1B g.-271C>A enzyme gene polymorphism in asthmatic children

This study indicated that there is significant difference between genetic polymorphism of OATP2B1 transporter genes c.935G>A and ACT results. There are significant differences (P value less than 0.05) between each genotype in both controlled and uncontrolled asthma groups C table 3-13) and individual with GG (wild homozygous) genotypes show better ACT results than GA (mutant heterozygous) and AA (mutant homozygous) genotypes.

In meta-analysis conducted by Zhang, *et al* (2014), montelukast considerably decreased the frequency of asthma attacks compared to

placebo; nevertheless, its effectiveness was still inferior to that of ICS or ICS-LABA when used as first line and add on therapies.

Research compared montelukast and low-dose budesonide inhalers for treating pediatrics with moderate asthma that show that in the recent four-week period the montelukast group had superior asthma control including lower percentage of asthmatic children who experienced difficulties more than twice a week, woke up at night or coughed at night, or needed relief treatment more than twice a week. Also, individuals who had an ACT score below 19 were less common in the montelukast group (Chen, *et al*.,2021).

There was no significant difference (p value ,0.05) between ACT and different genotypes of CYP2C8*1B g.-271C>A polymorphisms (table 3-18).

4.7. Serum Total Serum IgE level among different genotypes of OAT2B1 c.935G>A transporter gene and CYP2C8*1B g.-271C>A enzyme gene polymorphism in asthmatic children

The current study shows that the level of serum total IgE not lowered considerable after montelukat therapy and there was significant difference (P value <0.05) between the serum total IgE level among different genotypes groups of OATP2B1 transporter genes c.935G>A polymorphism in controlled and uncontrolled groups (table 3-14,3-15).

High total IgE levels were also seen in asthmatic patients receiving control therapy in a study by Qu X *et al*. (2018), who reported that the IgE level in asthmatic patients receiving treatment was noticeably greater than that in asthmatic controls. Another study reported that the blood IgE levels in asthmatic individuals were only reduced by high dosages of ICSs and montelukast. (Stelmach, *et al.*, 2005). As result, OATP2B1 transporter gene c.935 G>A may have an impact on serum total IgE levels.

As result, OATP2B1 transporter gene c.935 G>A may have an impact on IgE levels as opposed to CYP2C8*1B enzyme gene g.-271C>A polymorphism, which have been demonstrated to have no significant correlation with total IgE level.

4.8. Pulmonary Function Test (PFT) prevalence among different genotypes of OAT2B1 c.935G>A transporter gene and CYP2C8*1B g.-271C>A enzyme gene polymorphisms in asthmatic children

In many other research, montelukast observed to produce a substantial reduction in symptoms of asthma and the proportion of rescue-free days, along with better pulmonary function (average FEV1 values increased by 68% and more (Barnes, *et al.*,2001).

The current study shows that patient that have GG wild homozygous genotype show near normal PEF and FEV1 values in contrast to patients with GA or AA mutant genotypes and there is significant statistics difference between these three genotypes groups in both controlled and uncontrolled asthma.

This finding is in contrast to a Stelmach, *et al.* (2007)' study in which children that taking montelukast for four weeks, either as a monotherapy or in combination with budesonide, showed a rise in FEF25-75% values. The mentioned study explains their finding by that whereas inhaled medications, often rarely reach the lower airways, systemic medications, such as oral montelukast are able, as their inflammation plays a critical part in the asthma progress.

The current results are in agreement with a study done by Nieto, *et al. (*2006) which found that montelukast had a positive impact on children's small airways as evaluated by impulse oscillometry, but had no impact on the FEF25-75% parameter.

There was no significant correlation between PFT (PEF and FEV1) and CYP2C8*1B enzyme gene g.-271C>A polymorphisms in all genotype's groups (table 3-22).

4.9. Pearson's correlation between clinical parameters and different genotypes of OAT2B1 c.935G>A and CYP2C8*1B g.-271C>A polymorphism in asthmatic children

The findings of the current study indicate that the genetic polymorphism of OATP2B1 c.935G>A transporter gene polymorphism has a significant correlation (p value less than 0.05) with the total serum IgE level, and PEF but not with FEV1. Also, there was no correlation between clinical parameters (Serum Total IgE, FEV1, and PEF) with another genetic polymorphism CYP2C8*1B g.271C>A enzyme gene polymorphism.

This outcome is in agreed with a study by Tantisira *et al.* (2009) that found the OATP2B1, transporter SNP (rs12422149), causes a glutamine substitution at position 312 and influences the plasma levels of montelukast. Comparing heterogeneous individuals to those with the wild genotypes, montelukast levels are reduced by 30% (Tantisira, *et al.*, 2009).

Numerous studies have reported associations between c.935G>A and c.1457C>T, the two most prevalent OATP2B1 missense SNVs (global average allelic frequency of 17.6 and 8.6%, respectively), and the drug pharmacokinetics or response of OATP2B1 substrate drugs. In several investigations (Mougey *et al*., 2009; Mougey *et al*., 2011), people bearing the most prevalent OATP2B1 c.935G>A mutation (*3 allele), had lower montelukast plasma level than those who did not (Kim, *et al.*, 2013; Tapaninen *et al*., 2013).

The CYP2C8*1B enzyme gene polymorphism did not show any significant effect (P value less than 0.05) with any parameters.

Conclusions & Recommendations

4.10. Conclusions

Depending on the obtained results, the followings may be concluded:

11. Both genetics polymorphism of OATP2B1 transporter gene c.935G>A and CYP2C8*1B enzyme gene g.-271C>A was detected in a sample of Iraqi Population.
12. The mutant heterozygous of OATP2B1 transporter gene c.935G>A GA (45%) was more prevalence than CYP2C8*1B gene g.-271C>A CA (22%) in Iraqi children.
13. The most frequent genotype was wild homozygous GG (48%) for OATP2B1 transporter gene c.935G>A and wild homozygous CC (74%) for CYP2C8*1B gene g.-271C>A in asthmatic children.
14. The polymorphism of OATP2B1 transporter affects the response of asthmatic children to montelukast therapy and asthmatic children having wild GG genotype showed better improvement in lung function (FEV1, PEF) and clinical condition (ACT, serum total IgE level) after treatment in comparison with those having mutant AA and GA genotypes in mild to moderate asthma.
15. The CYP2C8*1B g.-271C>A genetics polymorphism was not significantly associated with montelukast responses despite its existence in Iraqi asthmatic patients.

4.11. Recommendations for Future Works

1. Considering other genes polymorphism effect on montelukast and or studying other drugs given as add on therapy in mild to moderate asthmatic children (e.g., like corticosteroids inhaler beclomethasone), and estimating its weak response since it may be genetically based.
2. A case- control cohort study of same genes or other related genes and evaluating their effectiveness in long term asthma control performance.
3. Study on larger sample size is recommended to further address the relationship between genotype of OATP2B1 c.935G>A and CYP2C8 g.-271C>A and therapeutic response to Montelukast in children with asthma.
4. The patients with mutant genotypes (GA and GG) are not recommended to used montelukast as maintenance therapy.

4.12. Limitations of the study

1. Pulmonary function test can be done only in children above 6 years and children under this age don't have any clinically effective tests like PFT, to monitoring drugs effects and so excluded in many studies.
2. The asthmatic patients that used montelukast as monotherapy was fewer than that of other add on montelukast therapy and patients' selection was difficult and take most of study time.
3. The time of our study was so short and we cannot take more samples or doings any tests before and after treatment and our results compare with normal laboratory values that determine by kits leaflets and not patients before treatment values.

References

References

Alahmadi, T. S., Banjari, M. A., & Alharbi, A. S. (2019). The prevalence of childhood asthma in Saudi Arabia. *International journal of pediatrics & adolescent medicine*, 6(2); pp. 74–77.

Alahmadi, T. S., Hegazi, M. A., Alsaedi, H., Hamadallah, H., Atwah, A. F., & et al., (2023). Prevalence and risk factors of asthma in children and adolescents in Rabigh, *Western Saudi Arabia. Children,* 10(2), 247.

Al-Allaf, L. I. K. (2023). Montelukast: A Review of Articles on the Experimental Level. Annals of the College of Medicine, Mosul, 45(2), 227-236.

Al-Azzam, N. and Elsalem, L., (2020). Leukotriene D4 role in allergic asthma pathogenesis from cellular and therapeutic perspectives. *Life Sciences*, 260; p.118452.

Al-Samarai MA, Al-Samarai AM, Al-Mishhadany kA (2009). Epidemiological characteristics of bronchial asthma among primary school children in Samara, Iraq. *Tikrit Medical Journal*, 6 (2); pp. 106 -110.

Al-Thamiri, D., Al-Kubaisy, W., & Ali, S. H. (2005). Asthma prevalence and severity among primary-school children in Baghdad. *Eastern Mediterranean health journal*, 11(1-2); pp. 79–86.

Altaş, U., Altaş, Z. M., Öz, F., & Özkars, M. Y. (2023). Evaluation of Neuropsychiatric Effects of Montelukast–Levocetirizine Combination Therapy in Children with Asthma and Allergic Rhinitis. *Children*, 10(8), 1301.

Alolayan, A. M. H., Alabeesy, M. S. Y., Alqabbani, A. A. A., Almutairi, A. J. F., et al. (2021). Interrelationship between body mass index and asthma in children suffering from asthma-analytical cross-sectional study. *European review for medical and pharmacological sciences*, 25(16); pp. 5208–5214.

Aquilante, C. L., Niemi, M., Gong, L., Altman, R. B., & Klein, T. E. (2013). PharmGKB summary: very important pharmacogene information for cytochrome P450, family 2, subfamily C, polypeptide 8. *Pharmacogenetics and genomics*, 23(12); pp. 721–728.

Atkins D and Leung DY (2004): diagnosis of allergic diseases. In "Nelson Textbook of Pediatrics" (EDS).

Bahrman RE, Kliegmen RM, and Jenson HB (2019), 21th edition, Philadelphia, chapter 169, pp. 1188-1192.

Backman, J. T., Filppula, A. M., Niemi, M., & Neuvonen, P. J. (2015). Role of Cytochrome P450 2C8 in Drug Metabolism and Interactions. *Pharmacological Reviews*, 68(1); pp.168–241.

Bahadur, N., Leathart, J.B., Mutch, E., Steimel-Crespi, D., Dunn, S.A., et al. (2002). CYP2C8 polymorphisms in Caucasians and their relationship with paclitaxel 6α-hydroxylase activity in human liver microsomes. *Biochemical pharmacology*, 64(11); pp.1579-1589.

Bahmani, A. H. A., Slob, E. M., Bloemsma, L. D., Brandstetter, S., Corcuera-Elosegui, P., Gorenjak, M., & Maitland-van der Zee, A. H. (2023). Medication use in uncontrolled pediatric asthma: results from the

SysPharmPediA study. *European Journal of Pharmaceutical Sciences*, 181, 106360.

Bankova, L.G., Dwyer, D.F., Yoshimoto, E., Ualiyeva, S., McGinty, J.W., Raff, H., et, al. (2018). The cysteinyl leukotriene 3 receptor regulates expansion of IL-25–producing airway brush cells leading to type 2 inflammation. *Science immunology*, 3(28); pp 9453.

Bankova, L.G., Lai, J., Yoshimoto, E., Boyce, J.A., Austen, K.F., Kanaoka, Y. and Barrett, N.A., (2016). Leukotriene E4 elicits respiratory epithelial cell mucin release through the G-protein–coupled receptor, GPR99. *Proceedings of the National Academy of Sciences*, 113(22); pp.6242-6247.

Barnes, N., Wei, L. X., Reiss, T. F., Leff, J. A., Shingo, S., Yu, C., & Edelman, J. M. (2001). Analysis of montelukast in mild persistent asthmatic patients with near-normal lung function. *Respiratory medicine*, 95(5); pp.379–386.

Banjari, M., Kano, Y., Almadani, S., Basakran, A., Al-Hindi, M. and Alahmadi, T., (2018). The relation between asthma control and quality of life in children. *International journal of pediatrics*, volume 2018.pp4-5.

Bel, E.H., (2004). Clinical phenotypes of asthma. *Current opinion in pulmonary medicine*, 10(1); pp.44-50.

Bian, S., Li, L., Wang, Z., Cui, L., Xu, Y., Guan, K., and et al., (2021). Neuropsychiatric side reactions of leukotriene receptor antagonist, antihistamine, and inhaled corticosteroid: A real-world analysis of the Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS). *World Allergy Organization Journal*, 14(10); p.100594.

Bhakta, N. R., McGowan, A., Ramsey, K. A., Borg, B., Kivastik, J., & et al., (2023). European Respiratory Society/American Thoracic Society technical statement: standardization of the measurement of lung volumes, 2023 update. European Respiratory Journal, 62(4).

Björkander, S., Klevebro, S., Hernandez-Pacheco, N., Kere, M., Ekström, S., & et al., (2023). Obese asthma phenotypes display distinct plasma biomarker profiles. *Clinical and Translational Allergy*, 13(3), e12238

Bleecker, E.R., Menzies-Gow, A.N., Price, D.B., Bourdin, A., et al, (2020). Systematic literature review of systemic corticosteroid uses for asthma management. *American journal of respiratory and critical care medicine,* 201(3); pp.276-293.

Boehmer, A. L., & Merkus, P. J. (2006). Asthma therapy for children under 5 years of age. *Current opinion in pulmonary medicine,* 12(1); pp34–41.

Brand, P.L., Caudri, D., Eber, E., Gaillard, E.A., Garcia-Marcos, L., et al., (2014). Classification and pharmacological treatment of preschool wheezing: changes since 2008. *European respiratory journal*, 43(4); pp.1172-1177.

Brannan, J. D., & Lougheed, M. D. (2012). Airway hyperresponsiveness in asthma: mechanisms, clinical significance, and treatment. Frontiers in physiology, 3, p.460.

Calapai, G., Casciaro, M., Miroddi, M., Calapai, F., Navarra, M. and Gangemi, S., (2014). Montelukast-induced adverse drug reactions: a review of case reports in the literature. *Pharmacology*, 94(1-2); pp.60-70.

Cardoso, J. d. O., Oliveira, R. V., Lu, J. B. L., & Desta, Z. (2015). In Vitro Metabolism of Montelukast by Cytochrome P450S and UDP-Glucuronosyltransferases. Drug Metabolism and Disposition, 43(12); pp. 1905–1916.

Castro-Rodriguez, J. A., Rodriguez-Martinez, C. E., & Ducharme, F. M. (2018). Daily inhaled corticosteroids or montelukast for preschoolers with asthma or recurrent wheezing: A systematic review. Pediatric pulmonology, 53(12); pp.1670–1677.

Chang, C. L., Ali, G. B., Pham, J., Dharmage, S. C., Lodge, C. J., Tang, M. L., & Lowe, A. J. (2023). Childhood body mass index trajectories and asthma and allergies: A systematic review. *Clinical & Experimental Allergy*, 53(9), 911-929.

Chapman, D. G., & Irvin, C. G. (2015). Mechanisms of airway hyper-responsiveness in asthma: the past, present and yet to come. Clinical and experimental allergy: *journal of the British Society for Allergy and Clinical Immunology*, 45(4); pp.706–719.

Chipps B. E. (2008). Asthma in infants and children. *Clinical cornerstone*, 8(4); pp.44–61.

Chauhan, B.F., Chartrand, C., Chroinin, M.N., Milan, S.J. and Ducharme, F.M., (2015). Addition of long-acting β2-agonists to inhaled corticosteroids for chronic asthma in children. *Cochrane Database of Systematic Reviews,* Issue (11).

Chen, Z. M., Zhao, D. Y., Xiang, L., & Hong, J. G. (2021). Treatment of pediatric mild persistent asthma with low-dose budesonide inhalation suspension vs. montelukast in China. *World journal of pediatrics:* WJP, 17(6); pp. 619–625.

Chong, J., Haran, C., Chauhan, B.F. and Asher, I., (2015). Intermittent inhaled corticosteroid therapy versus placebo for persistent asthma in children and adults. *Cochrane Database of Systematic Reviews*, (7). 1465-1858

Crooks, S.W. and Stockley, R.A., (1998). Leukotriene B4. *The international journal of biochemistry & cell biology*, 30(2); pp.173-178.

Dahlin A, Litonjua A, Lima JJ, et al. (2015). Genome-Wide Association Study Identifies Novel Pharmacogenomic Loci for Therapeutic Response to Montelukast in Asthma. *PLoS ONE*, 10(6).

Darawi, M. N., Ai-Vyrn, C., Ramasamy, K., Hua, P. P., Pin, T. M., & et al., (2013). Allele-specific polymerase chain reaction for the detection of Alzheimer's disease-related single nucleotide polymorphisms. *BMC medical genetics*, 14, 27.

Davidson, R., Roberts, S. E., Wotton, C. J., & Goldacre, M. J. (2010). Influence of maternal and perinatal factors on subsequent hospitalisation for asthma in children: evidence from the Oxford record linkage study. BMC pulmonary medicine, 10, 1-8.

Davila, I., Valero, A., Entrenas, L.M., Valveny, N. and Herráez, L., (2015). Relationship between serum total IgE and disease severity in patients with

allergic asthma in Spain. *Journal of investigational allergology & clinical immunology*, 25(2); pp.120-127.

De Benedictis, F. M., & Attanasi, M. (2016). Asthma in childhood. *European respiratory review: an official journal of the European Respiratory Society,* 25(139); pp. 41–47.

De Benedictis, F. M., Vaccher, S., & de Benedictis, D. (2008). Montelukast sodium for exercise-induced asthma. *Drugs of today (Barcelona, Spain: 1998*, 44(11); pp. 845–855.

De Groot, J. C., Ten Brinke, A., & Bel, E. H. (2015). Management of the patient with eosinophilic asthma: a new era begins. *ERJ open research*, 1(1); 00024-2015.

De Oliveira Cardoso, J., Oliveira, R. V., Lu, J. B. L., & Desta, Z. (2015). In vitro metabolism of montelukast by cytochrome P450s and UDP-glucuronosyltransferases. Drug Metabolism and Disposition, 43(12), 1905-1916.

Desta, Z. and Flockhart, D.A., (2017). Pharmacogenetics of drug metabolism. In Clinical and translational science, *Academic Press.* pp. 327-345.

Dexheimer, J., Chambers, P., & Johnson, L. (2016). Asthma-related emergency department use: current perspectives. *Open Access Emergency Medicine*, Volume 8; pp. 47–55.

Dixon, E.G., Rugg-Gunn, C.E., Sellick, V., Sinha, I.P. and Hawcutt, D.B., (2021). Adverse drug reactions of leukotriene receptor antagonists in children with asthma: a systematic review. BMJ Paediatrics Open, 5(1); p.10

Doeing, D. C., & Solway, J. (2013). Airway smooth muscle in the pathophysiology and treatment of asthma. Journal of applied physiology, 114(7); pp.834–843.

Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma., (2020). National Institute of Health: National Heart, Lung, and Blood Institute. Available from; https://www.nhlbi.nih.gov/health-topics/guidelines-for-diagnosis-management-of-asthma. Date of visit; 3 December 2022. pp..12-20.

Faisal A, Sameer O, Iman S (2009). Prevalence of asthma in Jordan: Comparison between Bedouins and urban schoolchildren using the International Study of Asthma and Allergies in Childhood phase III protocol. *Allergy and Asthma Proceedings*, 30(2); pp181-185.

Ferrante, G., Fasola, S., Malizia, V., Licari, A., Cilluffo, G., Piacentini, G., & et al. (2022). *Pharmacogenomics: A Step forward Precision Medicine in Childhood Asthma. Genes,* 13(4); p 599.

Filppula, A. M., Laitila, J., Neuvonen, P. J., & Backman, J. T. (2011). Reevaluation of the Microsomal Metabolism of Montelukast: Major Contribution by CYP2C8 at Clinically Relevant Concentrations. *Drug Metabolism and Disposition*, 39(5); pp. 904–911.

García-Menaya, J.M., Cordobés-Durán, C., García-Martín, E. and Agúndez, J.A., (2019). Pharmacogenetic factors affecting asthma treatment response. potential implications for drug therapy. *Frontiers in pharmacology*, 10; p.520.

Gao, P., & Zhou, C. (2023). Progress of three kinds of treatment for asthma. *Highlights in Science, Engineering and Technology*, 54, 225-232.

Ghimire, J. J., & Kabra, S. K. (2023). Long-term Treatment of Asthma. Essential Paediatric Pulmonology, 281.

Global Initiative for Asthma (GINA)., (2023). Global strategy for asthma management and prevention, Date of visit 3 December 2022, chapter 1,2,3;pp 20,34-39,46.

Gosens, R. and Gross, N., (2018). The mode of action of anticholinergics in asthma. *European Respiratory Journal*, 52(4). pp7

Graham, B. L., Steenbruggen, I., Miller, M. R., Barjaktarevic, I. Z., Cooper, B. G., Hall, et al. (2019). Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *American journal of respiratory and critical care medicine*, 200(8); pp. e70–e88.

Haeggström, J.Z., (2004). Leukotriene A4 hydrolase. In Handbook of Proteolytic enzymes, *Academic press,* pp. 323-328.

Hameed, R.M., Ahmed, M.M., Abood, H.A.A.N. and Hussein, A.M., (2019). To evaluate total serum immunoglobulin E level and factors that

effect on this level in Iraqi asthmatic children. *Biomedical and Biotechnology Research Journal (BBRJ)*, 3(4); pp.240-244.

Hamelmann, E., (2007). The rationale for treating allergic asthma with anti-IgE. *European Respiratory Review,* 16(104); pp.61-66.

Hossny, E., Rosario, N., Lee, B.W., Singh, M., El-Ghoneimy, D., Soh, J.Y. and et al., (2016). The use of inhaled corticosteroids in pediatric asthma: update. *World Allergy Organization Journal*, (9); p.26.

Hoste, E., Paquot, A., Panin, N., Horion, S., El Hamdaoui, H., and et al., (2023). Genetic polymorphisms in SLCO2B1 and ABCC1 conjointly modulate atorvastatin intracellular accumulation in HEK293 recombinant cell lines. *Therapeutic Drug Monitoring*, 45(3), 400-408.

Hunt, R., Sauna, Z. E., Ambudkar, S. V., Gottesman, M. M., & Kimchi-Sarfaty, C. (2009). Silent (synonymous) SNPs: should we care about them. *Methods in molecular biology* (Clifton, N.J.), 578; pp. 23–39.

Incecik, F., Onlen, Y., Sangun, O. and Akoglu, S., (2007). Probable montelukast-induced hepatotoxicity in a pediatric patient: case report. *Annals of Saudi Medicine*, 27(6); pp.462-463.

Ismail S, and Essawi M. (2012). Genetics polymorphism studies in humans. *Middle East Journal of Medical Genetics*. 2012;1(2):57-63.

Iqbal S, Oraka E, Chew GL, Flanders WD (2014). Association between birthplace and current asthma: the role of environment and acculturation. *American journal of public health,* 104(S1), S175S182.

Jacobson, G.A., Raidal, S., Hostrup, M., Calzetta, L., Wood-Baker, R., & et al., (2018). Long-acting β2-agonists in asthma: enantioselective safety studies are needed. *Drug Safety*, (41; pp.441-449.

Jafari, M., Sobhani, M., Eftekhari, K., Malekiantaghi, A., Gharagozlou, M., & Shafiei, A. (2023). The Effect of Oral Montelukast in Controlling Asthma Attacks in Children: A Randomized Double-blind Placebo Control Study. *Iranian Journal of Allergy, Asthma and Immunology*, 22(5), 413-419.

Jat, K. R., & Agarwal, S. (2023). Lung function tests in infants and children. *Indian Journal of Pediatrics*, 90(8), 790-797.

Kanaoka, Y., Maekawa, A. and Austen, K.F., (2013). Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. *Journal of Biological Chemistry,* 288(16); pp.10967-10972.

Kansen, H.M., Le, T.M., Uiterwaal, C.S., van Ewijk, B.E., et al, (2020). Prevalence and predictors of uncontrolled asthma in children referred for asthma and other atopic diseases. *Journal of asthma and allergy,* pp.67-75.

Katzung BG, Masters SB, Trevor AJ. (2012). Basic and Clinical Pharmacology “12th Edition”. The Mc Graw Hill Companies Inc. United States. Chapter 20. pp.343-344.

Kim, K.A., Joo, H.J., Lee, H.M. and Park, J.Y., (2013). SLCO2B1 genetic polymorphisms in a Korean population: pyrosequencing analyses and comprehensive comparison with other populations. *Molecular biology reports*, 40(7); pp.4211-4217.

Kim, M., Deacon, P., Tirona, R. G., Kim, R. B., Pin, C. L., Meyer Zu Schwabedissen, & et al., (2017). Characterization of OATP1B3 and OATP2B1 transporter expression in the islet of the adult human pancreas. *Histochemistry and cell biology*, 148(4); pp.345–357.

Kindla, J., Rau, T. T., Jung, R., Fasching, P. A., Strick, R., Stoehr, R., & et al., (2011). Expression and localization of the uptake transporters OATP2B1, OATP3A1 and OATP5A1 in non-malignant and malignant breast tissue. *Cancer biology & therapy,* 11(6); pp. 584–591.

Kinzi, J., Grube, M. and Zu Schwabedissen, H.E.M., (2021). OATP2B1–The underrated member of the organic anion transporting polypeptide family of drug transporters. Biochemical Pharmacology, 188, p.114534.

Kliegman, R.M., Geme III, J.W.S., Blum, N., Shah, S.S. and Tasker, R.C. eds., (2020). Nelson. Tratado de pediatría. Elsevier Health Sciences. 169, pp.1189-1193.

Kolmert, J., Gómez, C., Balgoma, D., Sjödin, M., Bood, J., Konradsen, J. R., & et al. (2021). Urinary Leukotriene $E_4$and Prostaglandin D_2 Metabolites Increase in Adult and Childhood Severe Asthma Characterized by Type 2 Inflammation. A Clinical Observational Study. *American journal of respiratory and critical care medicine,* 203(1); pp. 37–53.

Kovač, K., Dodig, S., Tješić-Drinković, D. and Raos, M., (2007). Correlation between asthma severity and serum IgE in asthmatic children sensitized to Dermatophagoides pteronyssinus. *Archives of medical research,* 38(1); pp. 99-105.

Krishnamoorthy, M., Mohd Noor, N., Mat Lazim, N., & Abdullah, B. (2020). Efficacy of Montelukast in Allergic Rhinitis Treatment: A *Systematic Review and Meta-Analysis*. Drugs, 80(17); pp. 1831–1851.

Kudo, M., Ishigatsubo, Y., & Aoki, I. (2013). Pathology of asthma. *Frontiers in microbiology*, (4), p. 263.

Laitinen, A. and Niemi, M., (2011). Frequencies of single-nucleotide polymorphisms of SLCO1A2, SLCO1B3 and SLCO2B1 genes in a Finnish population. *Basic & clinical pharmacology & toxicology*, 108(1); pp.9-13.

Lassila, T., Hokkanen, J., Aatsinki, S. M., Mattila, S., Turpeinen, M., & Tolonen, A. (2015). Toxicity of Carboxylic Acid-Containing Drugs: The Role of Acyl Migration and CoA Conjugation Investigated. *Chemical research in toxicology*, 28(12); pp. 2292–2303.

Lee, Y. J., & Kim, C. K. (2020). Montelukast use over the past 20 years: monitoring of its effects and safety issues. *Clinical and experimental paediatrics*, 63(10); pp. 376–381.

Levy, M. L., Bacharier, L. B., Bateman, E., Boulet, L. P., Brightling, C., et al (2023). Key recommendations for primary care from the 2022 Global Initiative for Asthma (GINA) update. *NPJ primary care respiratory medicine*, 33(1); p. 7.

Levy, M. L., Fletcher, M., Price, D. B., Hausen, T., Halbert, R. J., & Yawn, B. P. (2006). International Primary Care Respiratory Group (IPCRG) Guidelines: diagnosis of respiratory diseases in primary care. Primary care

respiratory journal: *journal of the General Practice Airways Group,* 15(1); pp. 20–34.

Li, Q., Wang, K., Shi, H.-Y., Wu, Y.-E., Zhou, Y., Kan, M., Zhao, W. (2019). Developmental Pharmacogenetics of SLCO2B1 on Montelukast Pharmacokinetics in Chinese Children. Drug Design, Development and Therapy, Volume 13; pp. 4405–4411.

Li, W., Iusuf, D., Sparidans, R. W., Wagenaar, E., Wang, Y., de Waart, D. R., & et al. (2023). Organic anion-transporting polypeptide 2B1 knockout and humanized mice; insights into the handling of bilirubin and drugs. *Pharmacological Research*, 190, 106724.

Liu, M. C., Hubbard, W. C., Proud, D., Stealey, B. A., Galli, S. J., Kagey-Sobotka, A., & et al., (1991). Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics. Cellular, mediator, and permeability changes. The American review of respiratory disease, 144(1); pp. 51–58.

Lundbäck B, Backman H, Lötvall J, Rönmark E (2016). Is asthma prevalence still increasing? Expert review of respiratory medicine, 10, 39-51.

Lupu, V. V., Jechel, E., Fotea, S., Morariu, I. D., Starcea, I. M., Azoicai, A., ... & Ioniuc, I. (2023). Current Approaches in the Multimodal Management of Asthma in Adolescents—From Pharmacology to Personalized Therapy. *Biomedicines*, 11(9), 2429.

Lo, C. W. H., Pathadka, S., Qin, S. X., Fung, L. W., Yan, V. K. C., Yiu, H. H. E., ... & Chan, E. W. Y. (2023). Neuropsychiatric events associated with montelukast in patients with asthma: a systematic review. *European Respiratory Review*, 32(169).

Maagdenberg, H., Vijverberg, S. J., Bierings, M. B., Carleton, B. C., Arets, H. G., de Boer, A., & et al., (2016). Pharmacogenomics in Pediatric Patients: Towards Personalized Medicine. *Pediatric drugs*, 18(4), 251–260.

Maneechotesuwan, K., Sujaritwongsanon, P. and Suthamsmai, T., (2011). IgE production in allergic asthmatic patients with different asthma control status. *Journal of the Medical Association of Thailand*, 93(1); p.71.

Maglione, M., Giannattasio, A., Pascarella, A., & Tipo, V. (2023). Pediatric Asthma: Where Has Montelukast Gone? *Applied Sciences*, 13(7), 4146.

McFeely, S. J., Wu, L., Ritchie, T. K., & Unadkat, J. (2019). Organic anion transporting polypeptide 2B1 - More than a glass-full of drug interactions. Pharmacology & therapeutics, 196; pp. 204–215.

Medwid, S., Price, H. R., Taylor, D. P., Mailloux, J., et al. (2021). Organic Anion Transporting Polypeptide 2B1 (OATP2B1) Genetic Variants: In Vitro Functional Characterization and Association with Circulating Concentrations of Endogenous Substrates. *Frontiers in pharmacology,* 12; p. 713567.

Meshram, D., Bhardwaj, K., Rathod, C., Mahady, G.B. and Soni, K.K., (2020). The role of leukotrienes inhibitors in the management of chronic

inflammatory diseases. *Recent Patents on Inflammation & Allergy Drug Discovery*, 14(1); pp.15-31.

Miligkos, M., Bannuru, R. R., Alkofide, H., Kher, S. R., Schmid, C. H., & Balk, E. M. (2015). Leukotriene-receptor antagonists versus placebo in the treatment of asthma in adults and adolescents: a systematic review and meta-analysis. *Annals of internal medicine*, 163(10); pp. 756–767.

Mohammad Y, Tabbah K, Mohammad S, et al. (2010). International Study of Asthma and Allergies in Childhood: phase 3 in the Syrian Arab Republic. *Eastern Mediterranean Health Journal*, 16(7), 710-716.

Moon, T. C., St Laurent, C. D., Morris, K. E., Marcet, C., Yoshimura, T., Sekar, Y., & Befus, A. D. (2009). Advances in mast cell biology: new understanding of heterogeneity and function. *Mucosal Immunology*, 3(2); pp. 111–128.

Moore, W.C., Meyers, D.A., Wenzel, S.E., Teague, W.G., Li, H., et al. (2010). Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *American journal of respiratory and critical care medicine,* 181(4); pp.315-323.

Murphy, A.G., Casey, R., Maguire, A., Tosetto, M., Butler, C.T., et al. (2016). Preclinical validation of the small molecule drug quininib as a novel therapeutic for colorectal cancer. *Scientific reports*, 6(1); p.34523.

Mougey, E. B., Feng, H., Castro, M., Irvin, C. G., & Lima, J. J. (2009). Absorption of montelukast is transporter mediated: a common variant of

OATP2B1 is associated with reduced plasma concentrations and poor response. Pharmacogenetics and genomics, 19(2), 129–138.

Mougey, E. B., Lang, J. E., Wen, X., & Lima, J. J. (2011). Effect of citrus juice and SLCO2B1 genotype on the pharmacokinetics of montelukast. *Journal of clinical pharmacology*, 51(5); pp. 751–760.

Murphy, K., Mahmood, N., Craven, D., Gallagher, J., Ross, K., & et al., (2020). Randomized pilot trial of ipratropium versus placebo in children with critical asthma. Pediatric Pulmonology, 55(12); pp.3287-3292.

Murphy, R. C., Hammarström, S., & Samuelsson, B. (2018). Pillars Article: Leukotriene C: A Slow-Reacting Substance from Murine Mastocytoma Cells. Proc. Natl. Acad. Sci. USA. 1979. 76: 4275-4279. *Journal of immunology (*Baltimore, Md. : 1950), 200(5);pp 1538–1542.

Nannini, L.J., Luhning, S., Rojas, R.A., Antunez, J.M., Miguel Reyes, J.L., Cano Salas, C. and Stelmach, R., (2021). Position statement: asthma in Latin America. IS short-acting beta-2 agonist helping or compromising asthma management. *Journal of Asthma*, 58(8); pp.991-994.

Narmetova, S. Y. (2024). Effectiveness of the drug L-montus (Montelukast) in the treatment of bronchial asthma. *Central Asian Journal of Medical and Natural Science*, 5(1), 489-492.

National Bethesda, M.D., (2007). Expert Panel Report 3: Guidelines for the Diagnosis andmanagement of Asthma. *NIH Publication,* 7; pp.4051-4057.

Nieto, A., Pamies, R., Oliver, F., Medina, A., Caballero, L., & Mazon, A. (2006). Montelukast improves pulmonary function measured by impulse oscillometry in children with asthma (Mio study). *Respiratory medicine,* 100(7); pp. 1180–1185.

Nwaru, B.I., Ekström, M., Hasvold, P., Wiklund, F., Telg, G. and Janson, C., (2020). Overuse of short-acting β2-agonists in asthma is associated with increased risk of exacerbation and mortality: a nationwide cohort study of the global SABINA programme. *European Respiratory Journal,* 55(4); pp 2-5

O'Byrne, P.M., Pedersen, S., Lamm, C.J., Tan, W.C. and Busse, W.W., (2009). Severe exacerbations and decline in lung function in asthma. *American journal of respiratory and critical care medicine*, 179(1); pp.19-24.

Ophir, J., Brenner, S. and Kivity, S., (1985). Leukotrienes. *International Journal of Dermatology*, 24(4); pp.199-203.

Paljarvi, T., Forton, J., Luciano, S., Herttua, K. and Fazel, S., (2022). Analysis of neuropsychiatric diagnoses after montelukast initiation. *JAMA Network Open,* 5(5); pp. e2213643-e2213643.

Patil, M. N., Datkhile, K. D., Gudur, A. K., Gudur, R. A., & Patil, S. R. (2023). Single-nucleotide polymorphism in CYP1A1, CYP1B1, CYP2B6, CYP2C8, and CYP2C9 genes and their association with gastrointestinal cancer: A hospital-based case-control study. *Journal of Cancer Research and Therapeutics*. p. 2.

Pesant, C., Santschi, M., Praud, J.-P., Geoffroy, M., Niyonsenga, T., & Vlachos-Mayer, H. (2007). Spirometric pulmonary function in 3- to 5-year-old children. *Pediatric Pulmonology*, 42(3); pp. 263–271.

Peters-Golden, M. and Brock, T.G., (2003). 5-lipoxygenase and FLAP. Prostaglandins, leukotrienes and essential fatty acids, *New England Journal of Medicine*,69(2-3); pp.99-109.

Peters-Golden, M., Gleason, M.M. and Togias, A., (2006). Cysteinyl leukotrienes: multi-functional mediators in allergic rhinitis. *Clinical & Experimental Allergy*, 36(6); pp.689-703.

Peters-Golden, M. and Henderson Jr, W.R., (2007). Leukotrienes. *New England Journal of Medicine,* 357(18); pp.1841-1854.

Petsky, H. L., Cates, C. J., Kew, K. M., & Chang, A. B. (2018). Tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils): a systematic review and meta-analysis. *Thorax*, 73(12); pp. 1110–1119.

Profita, M., Sala, A., Bonanno, A., Siena, L., Ferraro, M., et al. (2008). Cysteinyl leukotriene-1 receptor activation in a human bronchial epithelial cell line leads to signal transducer and activator of transcription 1-mediated eosinophil adhesion. *Journal of Pharmacology and Experimental Therapeutics,* 325(3); pp.1024-1030.

Qu, X., Chen, Y., & Yin, C. (2018). Effect of montelukast on the expression of $CD4^{+}CD25^{+}$regulatory T cells in children with acute bronchial asthma. *Experimental and therapeutic medicine*, 16(3); pp. 2381–2386.

Raissy, H.H., Kelly, H.W., Harkins, M. and Szefler, S.J., (2013). Inhaled corticosteroids in lung diseases. *American journal of respiratory and critical care medicine,* 187(8); pp.798-803.

Ramsay, C. F., Sullivan, P., Gizycki, M., Wang, D., Swern, A. S., & et al., (2009). Montelukast and bronchial inflammation in asthma: A randomized, double-blind placebo-controlled trial. *Respiratory Medicine,* 103(7); pp. 995–1003.

Ravasi, S., Citro, S., Viviani, B., Capra, V. and Rovati, G.E., (2006). CysLT 1 receptor-induced human airway smooth muscle cells proliferation requires ROS generation, EGF receptor transactivation and ERK1/2 phosphorylation. *Respiratory research,* 7; pp.1-18.

Reddy, M.B. and Covar, R.A., (2016). Asthma phenotypes in childhood. *Current opinion in allergy and clinical immunology,* 16(2); pp.127-134.

Ricciardolo, F. L., Guida, G., Bertolini, F., Di Stefano, A., & Carriero, V. (2023). Phenotype overlap in the natural history of asthma. *European Respiratory Review*, 32(168).

Rodriguez-Antona, C., Niemi, M., Backman, J.T., Kajosaari, L.I., et al. (2008). Characterization of novel CYP2C8 haplotypes and their contribution to paclitaxel and repaglinide metabolism. *The pharmacogenomics journal*, 8(4); pp.268-277.

Romero-Tapia SdJ, Becerril-Negrete JR, Castro-Rodriguez JA, Del-Río-Navarro BE (2023). Early Prediction of Asthma. *Journal of Clinical Medicine.* 12(16):5404.

Rovati, G.E. and Capra, V., (2007). Cysteinyl-leukotriene receptors and cellular signals. *The Scientific World Journal*, 7; pp.1375-1392.

Saini, J., Mazumder, A., & Khan, A. (2023). Traditional medicine-a gold mine in the treatment of asthma. 711-725.

Salazar A, Velázquez-Soto H, Ayala-Balboa J, et al. (2017) Allergen-Based Diagnostic: Novel and Old Methodologies with New Approaches. *Allergen. InTech.*

Sandeep, T., Roopakala, M.S., Silvia, C.R.W.D., Chandrashekara, S. and Rao, M., (2010). Evaluation of serum immunoglobulin E levels in bronchial asthma. *Lung India: official organ of Indian Chest Society,* 27(3); p.138.

Sánchez, G., & Buitrago, D. (2018). Effect of montelukast 10 mg in elderly patients with mild and moderate asthma compared with young adults. Results of a cohort study. The Open Respiratory Medicine Journal, 12, 67.

Satriyani, N., Mahmuda, H. and Hartienah, S.D., (2022). Pharmacogenomics of asthma in children. *Indonesian Journal of Pharmacology and Therapy*, 3(2).

Schatz, M., Kosinski, M., Yarlas, A.S., Hanlon, J., Watson, M.E. and Jhingran, P., (2009). The minimally important difference of the Asthma

Control Test. *Journal of Allergy and Clinical Immunology*, 124(4); pp.719-723.

Schiffers, C., Wouters, E. F., Breyer-Kohansal, R., Buhl, R., Pohl, W., Irvin, C. G., ... & Hartl, S. (2023). Asthma prevalence and phenotyping in the general population: the LEAD (lung, hEart, sociAl, boDy) study. *Journal of Asthma and Allergy*, 16; 367-382.

Serebrisky, D., & Wiznia, A. (2019). Pediatric Asthma: A Global Epidemic. *Annals of global health*, 85(1); p.6.

Singh, R.K., Gupta, S., Dastidar, S. and Ray, A., (2010). Cysteinyl leukotrienes and their receptors: molecular and functional characteristics. *Pharmacology*, 85(6); pp.336-349.

Sinyor, B. and Perez, L.C., (2022). Pathophysiology of asthma. In StatPearls Publishing.

Shehibi, N.M.A.A., Ibrahim, K.M.S., Salam, M.M.A. and Mohamed, R.M., (2021). Assessment of Response to Anti Leukotriene Among Children with Bronchial Asthma. *European Journal of Molecular & Clinical Medicine*, 8(3); pp.3000-3008.

Shipp, C. L., Gergen, P. J., Gern, J. E., Matsui, E. C., & Guilbert, T. W. (2023). Asthma Management in Children. *The journal of allergy and clinical immunology.* In practice, 11(1); pp. 9–18.

Sjöström, M., Johansson, A.S., Schröder, O., Qiu, H., Palmblad, J. and Haeggström, J.Z., (2003). Dominant expression of the CysLT2 receptor accounts for calcium signaling by cysteinyl leukotrienes in human umbilical

vein endothelial cells. *Arteriosclerosis, thrombosis, and vascular biology,* 23(8); pp. e37-e41.

Slejko, J.F., Ghushchyan, V.H., Sucher, B., Globe, D.R., Lin, S.L., & et al., (2014). Asthma control in the United States, 2008-2010: indicators of poor asthma control. *Journal of Allergy and Clinical Immunology*, 133(6); pp.1579-1587.

Stelmach, I., Bobrowska-Korzeniowska, M., Majak, P., Stelmach, W., & Kuna, P. (2005). The effect of montelukast and different doses of budesonide on IgE serum levels and clinical parameters in children with newly diagnosed asthma. *Pulmonary pharmacology & therapeutics,* 18(5); pp. 374–380.

Stelmach, I., Grzelewski, T., Bobrowska-Korzeniowska, M., Stelmach, P., & Kuna, P. (2007). A randomized, double-blind trial of the effect of anti-asthma treatment on lung function in children with asthma. Pulmonary pharmacology & therapeutics, 20(6); pp. 691–700.

Sun, W., & Liu, H. Y. (2019). Montelukast and Budesonide for Childhood Cough Variant Asthma. *Journal of the College of Physicians and Surgeons--Pakistan: JCPSP*, 29(4); pp. 345–348.

Tantisira, K.G. and Drazen, J.M., (2009). Genetics and pharmacogenetics of the leukotriene pathway. *Journal of Allergy and Clinical Immunology,* 124(3); pp.422-427.

Teder, T., König, S., Singh, R., Samuelsson, B., Werz, O., Garscha, U., & Haeggström, J. Z. (2023). Modulation of the 5-Lipoxygenase Pathway by

Chalcogen-Containing Inhibitors of Leukotriene A4 Hydrolase. International journal of molecular sciences, 24(8), 7539.

Tran, T. N., Khatry, D. B., Ke, X., Ward, C. K., & Gossage, D. (2014). High blood eosinophil count is associated with more frequent asthma attacks in asthma patients. Annals of allergy, asthma & immunology: *official publication of the American College of Allergy, Asthma, & Immunology,* 113(1); pp. 19–24.

Trinh, H. K. T., Lee, S. H., Cao, T. B. T., & Park, H. S. (2019). Asthma pharmacotherapy: an update on leukotriene treatments. *Expert review of respiratory medicine*, 13(12); pp. 1169–1178.

Tataurov, A. V., You, Y., & Owczarzy, R. (2008). Predicting ultraviolet spectrum of single stranded and double stranded deoxyribonucleic acids. *Biophysical chemistry*, 133(1-3); pp. 66–70.

Tsopanoglou, N.E., Pipili-Synetos, E. and Maragoudakis, M.E., (1994). Leukotrienes C4 and D4 promote angiogenesis via a receptor-mediated interaction. *European journal of pharmacology*, 258(1-2); pp.151-154.

Ungar, W.J., Hadioonzadeh, A., Najafzadeh, M., Tsao, N.W., Dell, S. and Lynd, L.D., (2015). Parents and adolescents' preferences for asthma control: a best-worst scaling choice experiment using an orthogonal main effects design. *BMC Pulmonary Medicine*, 15, pp.1-10.

Watson, S., Kaminsky, E., Taavola, H., Attalla, M., & Yue, Q. Y. (2022). Montelukast and Nightmares: Further Characterisation Using Data from VigiBase. Drug safety, 45(6); pp. 675–684.

Wenzel, S.E., (2012). Asthma phenotypes: the evolution from clinical to molecular approaches. *Nature medicine*, 18(5); pp.716-725.

Wermuth, H.R., Badri, T. and Takov, V., (2022). Montelukast. In StatPearls. StatPearls Publishing.

Whittlesea C., Hodson K., (2019). Clinical pharmacy and therapeutics. 6 ed., Elsevier Limited. p. 440.

Woodfork, K. (2023). Asthma and chronic obstructive pulmonary disease (COPD). Introduction to Clinical Pharmacology: From Symptoms to Treatment. p.110.

Wyatt, E.L., Borland, M.L., Doyle, S.K. and Geelhoed, G.C., (2015). Metered-dose inhaler ipratropium bromide in moderate acute asthma in children: a single-blinded randomised controlled trial. *Journal of paediatrics and child health*, 51(2); pp.192-198.

Yajima, K., Akiyoshi, T., Sakamoto, K., Suzuki, Y., Oka, T., Imaoka, A., ... & Ohtani, H. (2023). Determination of single-molecule transport activity of OATP2B1 by measuring the number of transporter molecules using electrophysiological approach. Journal of Pharmacological Sciences, 153(3), 153-160.

Yang K. D. (2005). Asthma management issues in infancy and childhood. *Treatments in respiratory medicine*, 4(1); pp. 9–20.

Yao, T.-C., Ou, L.-S., Yeh, K.-W., Lee, W.-I., Chen, L.-C., & Huang, J.-L. (2011). Associations of Age, Gender, and BMI with Prevalence of Allergic

Diseases in Children: PATCH Study. *Journal of Asthma*, 48(5); pp. 503–510.

Yawn, B. P., Brenneman, S. K., Allen-Ramey, F. C., Cabana, M. D., & Markson, L. E. (2006). Assessment of asthma severity and asthma control in children. Pediatrics, 118(1); pp. 322–329.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC bioinformatics*, 13, 134.

Ye, Q., He, X.O. and D'Urzo, A., (2017). A review on the safety and efficacy of inhaled corticosteroids in the management of asthma. *Pulmonary Therapy,* 3; pp.1-18.

Yi, L., Zhao, Y., Guo, Z., Li, Q., Zhang, G., Tian, X., ... & Luo, Z. (2023). The role of small airway function parameters in preschool asthmatic children. *BMC Pulmonary Medicine*, 23(1), 219.

Yılmaz, M., Ozic, C. and Gok, İ., (2012). Principles of nucleic acid separation by agarose gel electrophoresis. Gel Electrophoresis–Principles and Basics, 4; p.33.

Zeind C., G. Carvolho M., (2018). Applied therapeutic. Eleventh ed. p.377.
Zhang, H.P., Jia, C.E., Lv, Y., Gibson, P.G. and Wang, G., (2014), July. Montelukast for prevention and treatment of asthma exacerbations in adults: Systematic review and meta-analysis. *In Allergy Asthma Proc* (Vol. 35), No. 4, pp. 278-87.

Zhu, Z., Homer, R. J., Wang, Z., Chen, Q., Geba, G. P., et al. (1999). Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *The Journal of clinical investigat*ion, 103(6), pp. 779–788.

وزارة الصحة
دائرة صحة كربلاء
مركز التدريب والتنمية البشرية
لجنة البحوث

استمارة رقم ٢٠٢١/٠٣

رقم القرار ١٨٤

تاريخ القرار ٢٠٢٢/١٠/٢١

قرار لجنة البحوث

درست لجنة البحوث في دائرة صحة كربلاء مشروع البحث ذي الرقم (٢٠٢٢٠١٨٢/كربلاء) المعنون:

Effect of Genetic polymorphism of opOATP2B1 transporter and CYP2C8 metabolising enzyme on montelukast response in Asthmatic children

والمقدم من الباحث :- **(حسناء حيدر محمد)**

الى شعبة ادارة المعرفة / وحدة ادارة البحوث في مركز التدريب والتنمية البشرية في دائرة صحة كربلاء بتاريخ ٢٠٢٢/١٠/٣١ وقررت:

قبول مشروع البحث اعلاه كونه مستوفيا للمعايير المعتمدة في وزارة الصحة والخاصة بتنفيذ البحوث ولا مانع من تنفيذه في مؤسسات الدائرة.

الدكتور
نعيم [illegible]
مقرر لجنة البحوث
طبيب اختصاص
31/10/2022

المرفقات:

Choose an item.

ملاحظات:

- تم تخويل عضو لجنة البحوث (د.تقوى خضر عبد الكريم) او مقرر اللجنة (د.نعيم عبيد طلال) للتوقيع على هذا القرار استنادا الى النظام الداخلي للجنة البحوث.
- الموافقة تعني ان مشروع البحث قد استوفى المعايير الاخلاقية والعلمية لإجراء البحث والمعتمدة في وزارة الصحة، اما التنفيذ فيعتمد على التزام الباحث بتعليمات المؤسسة الصحية التي سينفذ فيها البحث.

الخلاصة

المقدمة : مونتيلوكاست، أحد مضادات مستقبلات السيستينيل ليوكوترين 1، معتمد لعلاج الربو الحاد والمزمن لدى البالغين والأطفال.

ارتبط تعدد أشكال الجيني (SNP) في ناقل OATP2B1، c.935G>A (rs12422149) ، وإنزيم CYP2C8*1B g.-271 (rs7909236) بانخفاض تركيز المونتيلوكاست في البلازما لدى مرضى الربو.

هدف الدراسة: تهدف الدراسة إلى دراسة تأثير تعدد الأشكال الجينية لـناقل OATP2B1 وأنزيم CYP2C8على استجابة علاج المونتيلوكاست لدى الأطفال المصابين بالربو.

المرضى و طرائق العمل: أجريت هذه الدراسة الرصدية المقطعية في المركز التخصصي لامراض الحساسية و الربو في مستشفى كربلاء التعليمي للأطفال، من نهاية تشرين الأول 2022 إلى نهاية أيلول 2023على مائة من الذكور والإناث المتراوحة أعمارهم بين 6 إلى 15 سنة والذين يتناولون علاج المونتيلوكاست بانتظام لفترة لا تقل عن شهر . تم قياس اختبار وظائف الرئة (PEF و(FEV1، واختبار السيطرة على الربو(ACT) ، اضافة الى قياس مستوى IgE في مصل الدم. تم إجراء تفاعل البلمرة المتسلسل (PCR)الخاص بالأليل للكشف عن الأنماط الجينية المختلفة للمرضى بعد استخراج الحمض النووي

النتائج: أظهرت النتائج تعدد الاشكال الجينيه لجين OATP2B1 c.935G>A, كان 48% من الجينات المتجانسة GG و45% من الجينات المتغايرة GA و7% من الجينات المتماثلة .AA تشير التقديرات إلى أن تعدد الأشكال هذا له ارتباط كبير باستجابة المونتيلوكاست. تم تقدير أن تعدد الأشكال هذا له ارتباط كبير مع الاستجابة لعلاج المونتيلوكاست والمرضى الذين لديهم النمط الجيني المتماثل GG، يظهرون استجابة أفضل للعلاج والتي تقيس الاستجابة من خلال تحسن في اختبار وظائف الرئة، وانخفاض مستوى Ig E ، وتحسين أعراض الربو في اختبار السيطرة على الربو (النتيجة ≤20) بالمقارنة مع الأنماط الجينية المتغايرة (GA وAA).

فيما يتعلق بـ تعدد الأشكال الجينة لأنزيم CYP2C8*1B g.-271C ، تظهر نتائج الدراسة الحالية عدم وجود ارتباط كبير بين تعدد أشكال CYP2C8*1B و الاستجابة لعلاج المونتيلوكاست. توزيع CYP2C8*1B g.-271C الجين كان على النحو التالي؛ 74 (74٪) لجينات المتجانسة CC، 22 (22٪) لجينات المتغايرة CA و 4 (4٪) لجينات المتماثلة AA.

الاستنتاجات: تعدد أشكال ناقل OATP2B1 يؤثر على استجابة الأطفال المصابين بالربو لعلاج المونتيلوكاست، وتبينت النتائج في الأطفال المصابون بالربو الذين لديهم النمط الجيني GG تحسنًا

في وظائف الرئة , انخفاض مستوى IgE ودرجة≤20 في اختبار السيطرة على الربو بعد العلاج بالمقارنة مع أولئك الذين لديهم أنماط الوراثية المتغايرة (AAوGA).

لم يكن تعدد الأشكال الجيني في انزيم CYP2C8*1B g.-271C>A مرتبطا بشكل كبير مع استجابة المرضى لعلاج المونتيلوكاست على الرغم من وجوده في مرضى الربو.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة

تأثير تعدد النمط الجيني ل OAT2B1 وTransporter CYP2C8 Metabolizing Enzyme على استجابة علاج المونتيلوكاست لدى الأطفال المصابين بالربو

رسالة مقدمة الى مجلس كلية الصيدلة / جامعة كربلاء
كجزء من متطلبات نيل درجة الماجستير في علم الادوية والسموم

بواسطة
حسناء حيدر محمد

بكالوريوس صيدلة (جامعة كربلاء –2016)

إشراف
أ.د. عدي عبدالرضا حسين
أ.د. حسن محمود موسى

٢٠٢٤ميلادي
١٤٤٥ هـجري