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Toll-Like Receptor 2 And 4 Polymorphisms in Asthmatic Children and Its Relation to Cardiac Function

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ABSTRACT
Bronchial asthma is a common chronic inflammatory disorder of the airways that results from both genetic and environmental factors and can affect cardiovascular system on the long run. Toll-like receptors (TLRs) are a class of proteins that recognize the microbial pathogen-associated molecular patterns in the environment and bridge the innate and acquired immune systems.TLR2 and TLR4 are believed to play an important role in the pathogenesis of asthma. Activated TLR2 and TLR4 can directly or indirectly affect the function of regulatory T cells, thus influencing the Th1/Th2 imbalance and reducing inflammatory responses so it is important to identify TLR2 and TLR4 polymorphisms in asthma and its relation to severity of asthma and cardiac function. Bronchial asthma affects many organs including the heart. The aim of this study to review the role of TLR2 and TLR4 polymorphisms in asthmatic children and its relation to cardiac function

Keywords: Asthma; Toll-Like Receptor; Cardiac Function; Children

INTRODUCTION

Asthma is a common and serious chronic disease that has a substantial burden on patients, their families and the community. It causes respiratory symptoms, limitation of activity, and attacks that sometimes require urgent health care and may be fatal **(1).** It is a heterogeneous, multifactorial disease with variable and mostly reversible respiratory airway spasm based on a chronic inflammatory reaction. The clinical picture (cough, rhonchus, wheezing, chest tightness, or shortness of breath) are changeable and correlated with expiratory flow restriction **(2)**.

Toll like receptors include 11 members in human. Several variants in them have been proved associated with asthma. However, the result still conflicting **(3)**. TLR2 and TLR4 are the most important members of them proved associated with asthma. Variation in innate immunity genes such as TLR2 and TLR4 may modulate responsiveness to LPS and other PAMPS and thus play a role in the development of atopy and respiratory disease. TLR4 functions predominantly as a receptor for LPS whereas TLR2 is involved in the recognition of multiple products of gram positive organisms, mycobacteria and yeast **(4)**.

Etiology and risk factors for asthma:

Genetic, environmental and host factors must be considered in studying the risk factors for asthma **(5)**. It is well known that asthma runs in families and children with positive family history are at increased risk of developing asthma. The inheritance of asthma does not follow simple mandelian inheritance because asthma is not caused by mutation in single gene, but rather asthma is a multifactorial disease caused by the interaction of genetic and environmental factors **(6)**. Genes for asthma susceptibility usually fall under one of 4

categories, innate immunity and immunoregulation (as CD14, HLA genes and TLR4), T-helper 2 (Th2)-cell differentiation and activity (e.g. IL4⁄ IL4R, IL13 and FCER1B), epithelial biology and mucosal immunity (as CCL-genes and FLG), and lung function, airway remodeling and asthma severity (as ADRB2and TNF) **(7).**

Risk factors in the prenatal period are multifactorial, including prenatal maternal smoking associated with early childhood wheezing. Prenatal maternal smoking is also associated with increased risks of food allergy **(8)**. Higher intake of fish or fish oil during pregnancy is associated with lower risk of atopic disease (specifically eczema and atopic wheeze) up to age 6 years **(9)**. Similarly, higher vitamin E and zinc levels prenatally have been associated with lower risk of development of wheeze up to age 5 years **(10)**. There is an association between prenatal maternal stress and the development of allergic diseases in offspring **(11)**. Activation of the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS): the offspring's HPA axis or ANS, when persistently activated, can push offspring's immune development toward being allergy-prone **(12)**.

Also, sex affects the development of asthma in a time-dependent manner, the incidence and prevalence of asthma are greater among boys than among girls, and before age 12 years, boys have more severe asthma than girls, with higher rates of admission to hospital, later girls are more common than boys after puberty **(13)**. Additionally, exclusive breastfeeding for at least 3 months was associated with lower rates of asthma between 2 and 5 years of age **(14)**.

Family size and the number and order of siblings may affect the risk of development of asthma **(15)**.Children of parents with lower socio-economic status have greater morbidity from asthma **(16)**. Postnatal exposure to environmental tobacco smoke, especially from maternal smoking, has been consistently associated with respiratory symptoms of wheezing **(17)**.

The effects of gene-by-environment interactions in asthma are complex. In some cases the genes code for enzymes that detoxify inhaled agents (e.g., glutathione transferase genes and environmental pollution), whereas in other cases, the exposures may have a more direct effect on gene expression via epigenetic mechanisms **(18)**.

The use of antibiotics has been associated with early wheezing and asthma in several studies **(19)**.Severe infection with certain viruses such as respiratory syncytial virus and rhinovirus may play a role in persistent wheezing, although other studies have suggested no effect **(20)**.

Total serum immunoglobulin E level, a surrogate for allergen sensitivity, has been associated with the incidence of asthma. High levels of immunoglobulin E at birth were associated with greater incidence of both atopy and aeroallergen sensitivity but not necessarily asthma **(21)**.

Additionally, gastroesophageal reflux and asthma are often encountered together, and complex interactions occur during which GERD may increase asthmatic symptoms or asthma may trigger or worsen GERD **(22)**.

A meta-analysis, including 6 prospective cohort studies on the effect of body weight on future risk of asthma, found a twofold increased risk in obese children compared with normal-

weight children, suggesting that obesity is an independent risk factor for childhood asthma **(23)**.

Likewise, factor that may trigger or worsen asthma symptoms includes viral infections, domestic or occupational allergens (e.g. house dust mite, pollens, and cockroach), tobacco smoke, exercise and stress. These responses are more likely when asthma is uncontrolled. Some drugs can induce or trigger asthma e.g. beta-blockers, and (in some patients), aspirin or other NSAIDs **(1)**.

Pathophysiology and Pathogenesis of Asthma:

Asthma is a common pulmonary condition defined by chronic inflammation, tightening of respiratory smooth muscle, and episodes of bronchoconstriction **(24)**. Airway narrowing and a subsequent interference with airflow is the dominant physiological event leading to clinical symptoms in asthma. In acute exacerbations of asthma, bronchoconstriction occurs quickly to narrow the airways in response to exposure to a variety of stimuli including allergens or irritants **(25)**. Bronchospasms, edema, excessive mucus and epithelial and muscle damage can lead to bronchoconstriction with bronchospasm. Sharp contractions of bronchial smooth muscle, bronchospasm causes the airways to narrow; edema from micro vascular leakage contributes to airway narrowing **(26)**.

Inhaled allergens activate sensitized mast cells by crosslinking surface-bound immunoglobulin (Ig) E molecules to release several bronchoconstrictor mediators, including cysteinyl leukotrienes (cys-LT) and prostaglandin D2 (PGD2). Epithelial cells release stem-cell factor (SCF), which is important for maintaining mucosal mast cells at the airway surface. Allergens are processed by myeloid dendritic cells, which are conditioned by thymic stromal lymphopoietin (TSLP) secreted by epithelial cells and mast cells to release the chemokines CC31 chemokine ligand (CCL)17 and CCL22, which act on CC-chemokine receptor 4 (CCR4) to attract T helper 2 (Th-2) cells. Th-2 cells have a central role in orchestrating the inflammatory response in allergy through the release of interleukin-4 (IL-4) and IL-13 & IL-5 which is necessary for eosinophilic inflammation and IL-9 which stimulates mast-cell proliferation **(27)**. Moreover, bradykinin has been implicated to contribute to allergic inflammation and the pathogenesis of allergic conditions **(28)**.

In asthmatic patients, there is an increase in the number of T-cells in the airways and these are predominantly T helper (Th) type 2 cells **(29)**.By secreting the cytokines IL-4 and IL-13, which drive immunoglobulin (Ig)E production by B-cells, IL-5, which is solely responsible for eosinophil differentiation in the bone marrow, and IL-9, which attracts and drives the differentiation of mast cells, Th-2 cells have a central role in allergic inflammation **(30)**.

Phenotypes of Asthma:

Asthma is a heterogeneous disease, with different underlying disease processes. Recognizable clusters of demographic, clinical and/or pathophysiological characteristics are often called 'asthma phenotypes' **(31)**. Phenotyping approaches support the existence of an early-onset, mostly atopic and allergic asthma phenotype, and additionally identified a later-onset eosinophilic phenotype **(32)**. In early-onset asthma, the amounts of total and allergen-specific IgE are also higher in early-onset asthma than in late-onset asthma. People who have atopic asthma have higher amounts of TH2 cytokines and greater numbers of cells with receptor bound IgE than people who have atopy but do not have asthma **(31)**. While, late-

onset asthmatic patients tend to be non-allergic, and often require higher doses of ICS or are relatively refractory to corticosteroid treatment **(34)**.

Studies have shown that patients with late-onset asthma have persistent airflow limitation, low lung function and rapid decline of lung function **(35)**.A family history of asthma is also less commonly observed than in early-onset disease, and the genetics of this phenotype have not been specifically studied. This phenotype is often severe from onset **(36)**.

Exercise-induced asthma (EIA) refers to asthma whose symptoms are experienced primarily after exercise. People with EIA often have mild asthma and experience reactive bronchoconstriction (a decline in FEV1 of 10–15%) **(31)**.

As well, obesity has been suggested to have a substantial role in the development, control and severity of asthma **(37)**. Leptin has been shown to increase the oxidative and inflammatory response of alveolar macrophages derived from overweight and obese asthmatics **(38).**

Virus-induced asthma is a phenotype which is characterized by a sudden onset and severe clinical course. Each viral infection may alter the course of preexisting asthma, or can affect the immune system and subsequently modify the susceptibility to allergen sensitization and asthma in childhood. RSV, influenza and parainfluenza viruses may affect the airways and may cause inflammation, increased airway responsiveness and obstruction **(39).**

Neutrophilic asthma seen in corticosteroid-treated patients. Corticosteroids inhibit neutrophil apoptosis and, in some settings, contribute to neutrophil activation, suggesting that corticosteroid treatment itself is likely to have some role in the development of neutrophilia **(31)**.

Diagnosis of asthma:

Making the diagnosis of asthma is based on identifying both a characteristic pattern of respiratory symptoms such as wheezing, shortness of breath (dyspnea), chest tightness or cough, and variable expiratory airflow limitation **(1)**. Commencement of respiratory symptoms in childhood, a history of allergic rhinitis or eczema, or a family history of asthma or allergy, increases the probability that the respiratory symptoms are due to asthma **(34)**.

Spirometry is the recommended method of measuring airflow limitation and reversibility to establish a diagnosis of asthma. Measurements of FEV1 and FVC are made during a forced expiratory maneuver using a spirometer **(40)**. A normal or high FEV1 in a patient with frequent respiratory symptoms prompts consideration of alternative causes for the symptoms; e.g. cardiac disease, or cough due to post-nasal drip or gastroesophageal reflux **(1)**.

For patients who have symptoms consistent with asthma but normal lung function, measuring airway responsiveness to direct airway challenges (e.g. inhaled methacholine or histamine) or indirect airway challenges (e.g. inhaled mannitol or an exercise challenge) may help establish a diagnosis of asthma **(34)**. The test results are usually expressed as the provocative concentration (or dose) of the agonist causing a given fall (often 15% or 20%) in FEV1. Recent guidelines on exercise-induced bronchoconstriction recommend 10% fall in FEV1 as the criterion for a positive exercise challenge; the authors also noted that a criterion of 15% would provide greater specificity **(41)**.

The fractional concentration of exhaled nitric oxide (FENO) can be measured in some centers. FENO is not specific for asthma; elevated FENO may also be found in conditions such as is increased in eosinophilic asthma but also FENO is elevated in non-smokers with eosinophilic asthma who are not taking ICS **(42)**.

Toll like receptors and Asthma:

Toll-like receptors (TLRs) are an important family of receptors that constitute the first line of defense system against microbes. They can recognize both invading pathogens and endogenous danger molecules released from dying cells and damaged tissues and play a key role in linking innate and adaptive **(43)**.

TLRs are type I transmembrane proteins with 20–27 extracellular leucine-rich repeats (LRR) for the recognition of PAMP/DAMP, transmembrane domains, and intracellular toll–interleukin 1(IL-1) receptor (TIR) domains required for the activation of downstream signal transduction pathways **(44)**. TLRs are expressed in different cells, including neutrophils, macrophages, dendritic cells, and T and B lymphocytes, but also epithelial and endothelial cells. TLRs 1, 2, 4, 5 and 6 are located on the cell surfaces **(45).** TLR1/2 was originally reported to recognize bacterial cell components, but there is currently accumulating evidence that these receptor dimers can also be activated by viruses, including the RSV and rhinoviruses **(46).**

A number of TLRs recognizes double stranded ribonucleic acid during virus replication, including the RSV and rhinovirus ribonucleic acid **(47).** The binding of ligands to TLR stimulates specific intracellular downstream signaling cascades that initiate host defense reactions **(48).** Such PAMP-PRR interactions lead to production of pro-inflammatory cytokines and type 1 interferon that orient immune responses to particular microbes **(49)**. The main function of TLRs is the ability to recognize numerous pathogens **(44).** Each cell of immune system contains a specific group of TLR that exert special functions in recognizing PAMP/DAMP and mediating immune responses **(50)**. TLR function in innate immunity is through the induction of antimicrobial activity and the production of inflammatory cytokines **(51).** Several studies have presented considerable evidences on the contribution of TLR signaling dysregulation to the development and progression of numerous diseases such as autoimmune, chronic inflammatory and infectious diseases **(44)**.

In atherosclerosis, TLR (2&4) suppression resulted in diminishing inflammation in mouse models of atherosclerosis **(52).** In hypertension, TLR4 has been well-documented to mediate inflammation in vasculature **(53).**

Although TLRs are essential elements in innate immune system and play a critical role in the host-defensive mechanism against microbes, overactivation of TLRs disrupts the immune homeostasis leading to excessive pro-inflammatory cytokines production that is no doubt involved in the pathogenesis of many autoimmune and inflammatory diseases. Thus, inhibition of TLR signaling pathways has been predicted to be an effective therapeutic strategy to suppress unwanted, disease-associated inflammatory responses **(44).** Environmental factors can increase the risk for asthma as shown for the exposure to environmental tobacco smoke prenatally or during childhood or decrease the asthma risk, as it has been observed in numerous studies for children raised on traditional farms **(54).**

The adaptive immune system, represented by T and B cells, has a well-known, direct impact on allergies. B cells, under the direction and tight control of T cells, produce immunoglobulin (Ig)E, a prerequisite for an allergic immune response. While these mechanisms and their role in allergy had long been known, increasing doubt exists, that the adaptive immune system is responsible for the initiation of the immune deviations that lead to the development of asthma **(55).** Rather, the adaptive immune system may be triggered to amplify immune signals toward an allergic response. An immune deviation may arise from the innate immune system at barrier interfaces. Thus, a competent innate immune system is the first prerequisite for the immunological integrity of the surface–environment interface of higher organisms; a competent and interactive adaptive immune system is the second **(56)**.

Genetic alterations in the toll-like receptor system and allergy development

A special program of the American National Institute of Health (NIH) was devoted to identify genetic variation in components of the innate immune system, with a special focus on TLR genes. Thus, asthma susceptibility might also be associated with variation in genes encoding components of innate immunity, making them biologically plausible candidate genes for asthma **(57).** TLR2 &4 are the most relevant to the onset of asthma and to the inflammatory responses underlying asthmatic exacerbations.TLR 4 detects Gram-negative bacteria through their lipopolysaccharides **(58)**. TLR2 plays a large role in recognizing Gram-positive bacteria **(59).**

Th2 cells, mast cells, and eosinophils are commonly associated with the innate and adaptive immune response in asthma **(60).** There cognition of allergens, such as house dust mites (HDM),can activate TLR4 and subsequently allergen-specific Th2 cells **(58).**TLR2 promotes Th2-biased immune responses, which may be correlated to the Th1/Th2 imbalance in asthma. There are two distinct pathways in TLR signaling: myeloid differentiation factor 88 (MyD88)-dependent and independent.Both pathways are crucial in regard to the innate immune response. My D88 &Toll/IL-1 receptor-domain containing adapter-inducing interferon-b (TRIF) bind independently to TLRs, leading to the release of cytokines **(61)**.

During acute asthmatic exacerbations, the cleavage products of proteinases, such as fibrinogen, bind to TLR4s that are present in both the airway epithelium and macrophages, and the binding of these products results in allergic inflammation **(62).** In addition, asthmatic patients who ultimately die have increased expressions of TLR2, and TLR4, suggesting their potential role in the development of severe or even fatal asthmatic exacerbations **(63).** TLRs in asthma more or less susceptible to a specific disease **(64)**. Genetic polymorphisms affect the susceptibility, severity, and responsiveness of asthmatic patients to specific allergens. Variants of the TLR4 gene may either increase or decrease the sensitivity of the receptor to allergens **(65).**

Cardiovascular Risks and Interactions with TLR

Cardiovascular risk factors, notably diabetes, obesity, and insulin resistance, are also associated with a low-grade inflammatory state that reflects activation of innate immunity associated with metabolic and genetic factors **(53).**

There are an increasing number of studies demonstrating a major role of TLR in several animal models of ischemia reperfusion (I/R) injury. Cardiac I/R injury have a significant clinical relevance as, for example, in heart transplantation (HTx), myocardial infarction (MI), or coronary artery bypass graft surgery. Tissue damage and inflammation occurs after coronary

artery occlusion (ischemia) when reperfusion occurs **(66).** Cardiac I/R injury lead to the activation of multiple inflammatory pathways. Furthermore, there is an active interplay between pathways such as TLR and complement, resulting in complement activation and tissue damage **(67).**

A potential role of TLR2 in the response to oxidative stress has been established in neonatal rat cardiac myocytes in vitro. The TLR signaling cascade also seems to be important in cardiac hypertrophy. Several downstream proteins in the TLR cascade influence the development of cardiac hypertrophy **(68).**

Cardiac function and asthma

Bronchial asthma may be complicated by an increase in pulmonary vascular resistance, which consequently leads to secondary pulmonary hypertension and right ventricle (RV) dysfunction, caused by several mechanisms. The release of inflammatory mediators due to chronic inflammation of the airway may cause pulmonary vasoconstriction and distortion of pulmonary vasculature **(69)**.

The left ventricle (LV) could be affected secondary to RV involvement due to ventricular interdependence. Bronchial asthma may be complicated by an increase in pulmonary vascular resistance, which consequently leads to secondary,pulmonary hypertension and RV dysfunction caused by several mechanisms **(70)**.

The release of inflammatory mediators due to chronic inflammation of the airway may cause pulmonary vasoconstriction and distortion of pulmonary vasculature. There are many explanations for the occurrence of cardiac dysfunction in asthmatic children **(71)**.

Approach for adjusting asthma treatment:

The adjustment of asthma therapy is based on asthma control, and follows a step-up/step-down algorithm to increase or reduce the medication. Regular follow-up should occur in a period of 2–3 months to optimize the treatment strategy. It is important to record symptom control, lung function, risk factors, inhalation technique, adherence, and non-pharmacological strategies on a regular basis **(72)**.

Controller medications for children include inhaled corticosteroids (ICS), combination ICS/long-acting beta2-agonists (ICS/LABA), leukotriene receptor antagonists (LTRA) and chromones **(34)**.

Short-acting beta-agonists (SABA) are the most effective bronchodilators available, and therefore the preferred treatment for acute asthma in children of all ages. The inhaled route results in more rapid bronchodilation at a lower dose and with fewer side effects than oral or intravenous administration **(73)**.

Anti-IgE (omalizumab) has proven effect in children with moderate-to-severe and severe persistent allergic (IgE mediated) asthma. Anti-IgE treatment was associated with a significantly lower exacerbation rate, lower incidence of serious adverse events and there were significant improvements in quality of life in the patients receiving anti-IgE, both during stable ICS dosing and during tapering **(74)**.

The therapeutic range of plasma theophylline levels is 55–110 umol/L (5–10 mcg/ml), and measurement of plasma theophylline levels is recommended in otherwise healthy children when daily doses exceed 10 mg/kg/day **(75)**.

In children, as in adults, maintenance treatment with ICS controls asthma symptoms, reduces the frequency of acute exacerbations, the need for additional asthma medication and the number of hospital admissions, improves quality of life, lung function, and bronchial hyper responsiveness, and reduces exercise induced bronchoconstriction. When corticosteroid treatment is discontinued, asthma control deteriorates within weeks to months **(76)**.

CONCLUSION

TLRs are expressed on hematopoietic and non-hematopoietic airway cells, which play an immune-modulatory role in the development of asthma when activated by TLRs agonists. Due to the involvement of TLRs in innate and adaptive immunity, these receptors are currently being used as possible targets for drug development.

TLRs were significantly associated with asthma risk, severity and affection of cardiac function . Therefore, understanding the mechanisms and ways in which TLRs (2 & 4) are involved in the pathogenesis of asthma may suggest new strategies for controlling the disease and prevent cardiac complications and its early detection and managment.

No Conflict of interest.

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