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Association of Toll-Like Receptor (TLR) Gene Polymorphisms with Asthma: A Review

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ABSTRACT

Asthma and allergy are complex conditions often present in the same family or closely related subjects. Genetic factors undoubtedly contribute to disease susceptibility but the expression of the disease can be modulated by environmental exposures and the interactions between the two. Candidate-gene and linkage studies followed by positional cloning have already provided a large number of susceptibility genes. Toll-like receptors (TLRs) are a family of intracellular and cell surface receptors capable of responding to pathogen associated molecular patterns involved in the pathogenesis of asthma. Numerous epidemiological studies have demonstrated that single nucleotide polymorphisms (SNPs) in TLR genes have been reported to be associated with asthma risk. In this review, attempt has been made to discuss the findings of various studies conducted on human populations to evaluate the association of TLR gene polymorphisms with asthma or asthma related phenotypes.

KEY WORDS: asthma, toll-like receptor, gene, polymorphisms, association

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INTRODUCTION

Asthma is a chronic disorder, characterized by recurrent symptoms of wheeze, breathlessness, chest tightness and cough, often associated with bronchial hyperresponsiveness (BHR), variable airflow limitation and chronic inflammation of the airways[1]. It usually begins in childhood, often in association with an inherited susceptibility to produce IgE to common environmental allergens, including house dustmite, animal protein, fungal spores, and pollens[2]. It is estimated that up to 80% of children with asthma may be atopic. Atopy is a personal or familial tendency to become sensitized and produce IgE antibodies in response to common environmental allergen exposures[1]. It is estimated that approximately, 300 million people are affected worldwide causing 250 000 annual deaths by this airway disease[3]. The impact of bacterial products and their relationship to the development of asthma is increasingly a focus of interest and forms part of the so called "hygiene hypothesis". Exposure to farming environment in early life has been associated with a reduced risk of asthma and allergy in children compared to those who have not grown up on a farm[4,5].

Immune responses against pathogens are initiated by TLRs which have a critical role in both innate and adaptive immune responses. TLRs recognize invading microbes such as bacteria, viruses, parasites and fungi since these micro-organisms possess common structures called pathogen associated microbial patterns (PAMPs)[6,7]. TLRs and their ligands play an important role in antigen presentation and in immune modulation through activation of dendritic cells and regulating the immune response towards Th1, Th2 or Treg immunity[8]. For these reasons, the use of various TLR ligands in immunotherapeutic studies of allergic diseases such as asthma has attracted considerable interest in recent years. In this review we intend to discuss various TLRs and the findings of the association studies of TLR gene polymorphisms with asthma and related phenotypes.

1. TOLL LIKE RECEPTORS (TLRs)

The Toll receptors were first described in Drosophila as a transmembrane receptors responsible for determining the dorso-ventral polarity in the developing embryo[9]. There is a remarkable similarity in the signaling pathway of Drosophila Toll and the mammalian IL-1 pathway, leading to activation of NF-κB, a transcription factor responsible for the various aspects of inflammatory and immune responses. The cytoplasmic domains of Drosophila Toll and the mammalian IL-1 receptor are highly conserved and are referred to as the Toll/IL-1 receptor (TIR) domain[10]. A homologous family of Drosophila Toll receptors exists in vertebrates which is called Toll-like receptors (TLRs)[11]. TLRs are type I integral membrane glycoproteins with a cytoplasmic signaling domain and extracellular domains. To date, 13 TLRs (TLR1-TLR13) have been identified in humans and mice that are fundamental in recognizing the pathogen associated molecular patterns

(PAMPs)[12,13]. TLR11, TLR12 and TLR 13 only seem to be expressed in mice. The presence of a leucinerich repeat (LRR) domain in their extracellular domain and a TIR domain in their intracellular domain are the characteristic structural features of the members of TLR family. Based on the amino acid sequences of the human TLRs, the members of the TLR family can be divided into five subfamilies: the TLR3, TLR4, TLR5, TLR2 and TLR9 subfamilies. The TLR2 subfamily is composed of TLR1, TLR2, TLR6 and TLR10, the TLR9 subfamily is composed of TLR7, TLR8, and TLR9[10]. Depending upon the cellular localization, there are two subfamilies of TLRs. One subfamily (consisting of TLR1, 2, 4, 5, 6 and 11) is expressed on the cell surface and recognizes the various pathogen associated structures. On the other hand, the other subfamily (consisting of TLR3, 7, 8 and 9) is localized in the endoplasmic reticulum and endosomes or lysosomes and recognizes the nucleic acid of the pathogen[14].

1.1. TLR2 subfamily: TLR1, TLR2, TLR6 and TLR10

TLR2 is expressed as both a homodimer and a heterodimer associated with either TLR1 or TLR6. It is expressed on monocytes, macrophages and myeloid DC, and can bind a wide range of ligands, including lipoteichoic acid from Gram-positive bacteria, bacterial lipopeptides and glycolipids, fungal beta glucan (zymosan) and the endogenous DAMPs Hyaluronan, HSP70 and HMGB[15]. The recognition of lipoproteins from a wide range of organisms, including *Borrelia burgdorferi*, *Treponema pallidum*, *Aspergillus fumigatus* and *Mycoplasma fermentes*, has been attributed to TLR2[16]. In addition, it has also been reported that the viral proteins of Herpes Simplex Virus -1 and -2 (HSV-1 and HSV-2) Cytomegalovirus (CMV) are recognized by TLR2[17,18]. A common synthetic ligand of TLR2 is a triacylated lipopeptide Pam3CysSK4 (also known as Pam3Cys), which is a synthetic lipopeptide analogue of bacterial lipopeptide.

Like other members of the TLR family, *TLR10* contains a signal peptide, multiple (n=12) leucine-rich repeats, a cysteine-rich domain, a transmembrane domain, and a cytoplasmic Toll interleukin-1 receptor domain[19]. Although the ligand and the specific functions of human *TLR10* are not currently known, it is predominantly expressed in immune cell–rich tissues, including spleen, lymph node, and lung[20]. *TLR10* expression is barely detectable in naive human Bcells, but is rapidly induced after B-cell receptor triggering[21].

1.2. TLR3

The expression of TLR3 occurs in dendritic cells and B cells where they bind double stranded (viral) RNA and the endogenous microtubule regulator stathmin[15]. During the replication cycle of ssRNA or DNA viruses, the dsRNA is generated as an intermediate[22]. The dsRNA appears to be a

universal viral PAMP and TLR3 is considered to be the key receptor in an antiviral immune response[14]. The synthetic ligand of TLR3 is polyinosine-polycytidilic acid (polyI:C).

1.3. TLR 4

Lipopolysaccharide (LPS), composed of lipid A (endotoxin), core oligosaccharide, and O-antigen, the component of the outer membrane of the gram-negative bacteria, is recognized by TLR4. A complex formed by TLR4, MD2, and CD14 on various cells, such as macrophages and dendritic cells, is required for LPS recognition[23]. LPS associates first with LPS binding protein (LBP) and then with CD14, a glycosylphosphatidylinositol (GPI) anchored protein[24]. This complex binds to MD2 and associates with TLR4 which leads to its aggregation and subsequent signaling[23,25].

1.4. TLR5

The expression of TLR5 is high in the gut, particularly in lamina propria dendritic cells[26] where it controls the composition of the microbiota[27]. It recognizes a protein PAMP, i.e. bacterial flagellin[28]. The conserved D1 domain of flagellin has been identified as the site recognized by TLR5 by the mutational analyses of flagellin[29].

1.5. TLR9 subfamily: TLR7, TLR8 and TLR9

TLR7 and/or TLR8 recognize the single-stranded (ss) guanosine or uridine rich RNA and ssRNA viruses such as influenza, VSV, Newcastle disease virus (NDV)[30-33]. The synthetic antiviral nucleoside analogs such as imidazoquinolines (R848 or imiquimod) or loxoribine (7-allyl-7,8-dihydro-8-oxoguanosine) are the ligands for both TLR7 and TLR8[34-37]. Bacterial and viral DNA containing unmethylated CpG motifs are recognized by TLR9. The reduced stimulatory activity of CpG motifs in vertebrates is due to the greater extent of methylation and reduced frequency of CpG dinucleotide motifs. In contrast, CpG dinucleotides are non-methylated and occur in high frequency in viruses and bacteria[38]. CpG motifs have the potential to directly stimulate B cells and plasmacytoid dendritic cells (pDCs) and promote the production of type I interferons (IFNs), Th1-inducing cytokines and chemokines and co-stimulatory molecules[39].

2. ASSOCIATION OF TLR GENE POLYMORPHISMS WITH ASTHMA

It has been observed that the pathogenesis of asthma is regulated by the genetic variation within genes encoding TLRs. Several studies have been conducted to demonstrate the association of TLR gene polymorphisms with asthma in various populations across the world. Majority of the studies have investigated the association of TLR4 and TLR2 gene polymorphisms. However, some

studies have also evaluated the other TLR gene polymorphisms (TLR1, TLR3, TLR5, TLR6, TLR7, TLR8, TLR9 and TLR10) in asthma.

2.1. Association of TLR2 Gene polymorphisms

The TLR2/1596 C allele was reported to be associated with an increased risk for asthma in a case-control study and the family-based analysis[40]. The heterozygous (AG) genotype of TLR2Arg753Gln polymorphism was reported to be significantly increased among the asthmatic patients (62.5%) as compared to the controls (15%) (OR =9.4, 95% CI: 2.4-37.7) while the frequency of homozygous (GG) genotype of TLR2Arg753Gln polymorphism was increased among the controls (80%) as compared to the asthmatic patients (30%)[41]. In a case-control study comprising of 62 asthmatic and 61 controls of Puerto Rican population, the TLR2 +596 SNP was reported to be significantly associated with asthma (OR = 3.24 for CT, 2.71 for TT)[42]. However, in a case control study comprising of 318 asthmatic patients and 352 nonasthmatic controls from Chinese population, Qian et al. investigated the eight single-nucleotide polymorphisms in *TLR2* subfamily genes. They reported that patients with *TLR2*/rs7656411 TT variant homozygote had a significantly reduced risk of asthma when compared with those with the GG wild-type homozygote (OR= 0.63; 95% CI= 0.41-0.98; p=.036)[43]. Moreover, in an another case-control study conducted in an Iranian population, no association of TLR2 Arg753Gln polymorphism was reported with asthma or asthma features such as IgE levels, asthma history and pulmonary factors[44].

2.2. Association of TLR4 gene polymorphisms

Allele G (i.e. genotype AG or GG) of TLR4 gene had been shown to confer significantly increased risk for asthma in female subjects[45]. The frequency of heterozygous (AG) genotype of TLR4Asp299Gly polymorphism was significantly increased among the asthmatic patients (65%) as compared to the controls (30%) (OR =4.3, 95% CI: 1.4-13.8) while the homozygous (AA) genotype was reported to have increased frequency among the controls (70%) as compared to the asthmatics (20%)[41]. The results of a case-control study showed that the G allele of TLR-4 (Asp299Gly) polymorphism conferred significantly greater risk of asthma in early-onset asthma patients whereas the frequency of the G allele was not increased in late-onset asthma[46]. In a case-control study conducted in a Chinese Han population, the TT genotype of rs1927914 and the GG genotype of rs10983755 and rs1927907 of TLR4 gene polymorphisms were positively associated with asthma severity but no association was reported between TLR4 SNPs and asthma susceptibility[47]. The findings of an observational, cross-sectional study revealed that the asthmatic patients with high total serum IgE had a higher percentage of macrophages expressing TLR4 as compared to asthmatic

patients with normal total serum IgE (42.99 % ± 22.49 versus 28.84 % ± 15.16, p= 0.048). Weak correlation was reported between the percentage of macrophages expressing TLR4 in induced sputum and the total serum IgE level (R = 0.314; P = 0.040)[48].

In contrast, the findings of a case-control study revealed no association of TLR4 Asp299Gly polymorphism with asthma or asthma features such as IgE levels, asthma history and pulmonary factors[44]. In an another case-control study conducted to investigate the association of CD-14-159, TLR4-299 and TLR4-399 polymorphisms with asthma phenotypes, the TLR4-299 and TLR4-399 genotypes were shown to be not significantly associated with asthma phenotypes[49]. Another study conducted in North Indian population comprising of 481 asthma patients and 483 healthy controls revealed that the heterozygous genotype and the mutant (T) allele of the TLR4 C>1196T (Thr399Ile) polymorphism conferred resistance to asthma. No association was reported between the TLR4 A>896G (Asp299Gly) polymorphism and asthma[50].

2.3. Association of other TLR genes

TLR1 rs5743618 polymorphism was demonstrated to be associated with asthma and atopic eczema during the first 6 years of life after early bronchiolitis[51]. In a nested case-control study comprising of 624 asthmatic children and 1248 healthy controls, the protective effect of SNP in TLR1 gene on atopic asthma (OR= 0.54; 95% CI, 0.37-0.81; P 5 .002) was reported[52]. The results of a case-control study conducted in a Chinese Han population showed no association of *TLR3* SNPs with asthma susceptibility or asthma severity but the genetic variants in *TLR3* were associated with asthma-related phenotypes, including eosinophil counts, serum immunoglobulin E levels and lung function[53]. A positive association between the T allele of rs2381289 in *TLR6* and allergic rhinitis in asthma patients was reported. However, no association of this polymorphism with risk of asthma was reported in the study[43]. In a hospital-based study, conducted in 133 children hospitalized for bronchiolitis at <6 months of age, demonstrated that TLR6 rs5743810 was associated with present atopy at preschool age[51]. No association between the variants in TLR7 and TLR*8* genes and asthma susceptibility was reported in a case-control study conducted in a Chinese Han population. However, the genetic variants in TLR7 and 8 were shown to be associated with asthma-related phenotypes, including eosinophil counts, serum immunoglobulin E levels, lung function, and asthma severity[54]. In a study conducted by Törmänen et al., asthma ever was reported to be more common in girls (34.6%) having the TLR7 variant AT or TT genotype compared to those with homozygous for the major allele A (12.5%). It was further reported that the *TLR8* genotype was wild (GG) in 34.8% and variant (GC or CC) in 65.2% of the cases. No significant association was reported for the *TLR8* genotypes and asthma ever, current asthma, current atopic dermatitis, or current allergic rhinitis in

either girls or boys[55]. The *TLR9* genotype was wild (TT) in 32.1% and variant (TC or CC) in 67.9% of the cases. There were no significant associations between the *TLR3*, *TLR4*, or *TLR9* genotypes and asthma ever, current asthma, current atopic dermatitis, or current allergic rhinitis[55]. The SNPs in TLR9 gene along with CD14, TLR2, and TLR4 genes have been reported to modify the associations between country living and asthma[40]. The findings of a study showed the high frequency of AA genotype of *TLR10* gene polymorphism and low frequency of AG or GG genotypes (84.3% vs. 16.7%) in the cases with post-bronchiolitis asthma. It was shown that 30.0% of the children with variant AG or GG genotype had current asthma compared to 10.6% of those with homozygous for the major allele A. However, there were no statistically significant associations between *TLR10* gene polymorphisms and asthma ever, current atopic dermatitis, or current allergic rhinitis[55]. However, protective effects on atopic asthma was reported for single nucleotide polymorphisms in TLR10 gene (OR, 0.58; 95% CI, 0.39-0.86; P5.006)[52].

CONCLUSION

The findings of the epidemiologic studies on association of TLR gene polymorphisms with asthma and related phenotypes are inconsistent. Some studies have reported the significant association of TLR gene polymorphisms with asthma and related phenotypes while some studies have reported no association. These inconsistent findings may be mainly due to relatively small sample size and the effect of confounders. Further studies with larger sample size and prospective study are needed to confirm the putative associations of genetic variants in TLR genes with asthma.

CONFLICT OF INTEREST: None

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