Potential therapeutic targets from genetic and epigenetic approaches for asthma

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Author contributions: The author contributed to this paper with conception and literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Conflict-of-interest statement: There are no known conflicts of interest arising from this review.

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Received: August 5, 2015
Peer-review started: August 7, 2015
First decision: November 3, 2015
Revised: November 17, 2015
Accepted: December 29, 2015
Article in press: January 4, 2016
Published online: April 12, 2016

Abstract

Asthma is a complex disorder characterised by inflammation of airway and symptoms of wheeze and shortness of breath. Allergic asthma, atopic dermatitis and allergic rhinitis are immunoglobulin E (IgE) related diseases. Current therapies targeting asthma rely on non-specific medication to control airway inflammation and prevent symptoms. Severe asthma remains difficult to treat. Genetic and genomic approaches of asthma and IgE identified many novel loci underling the disease pathophysiology. Recent epigenetic approaches also revealed the insights of DNA methylation and chromatin modification on histones in asthma and IgE. More than 30 microRNAs have been identified to have regulating roles in asthma. Understanding the pathways of the novel genetic loci and epigenetic elements in asthma and IgE will provide new therapeutic means for clinical management of the disease in future.

Key words: Asthma; Immunoglobulin E; Genome-wide association studies; Epigenetics; MicroRNA

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Core tip: Asthma is a complex disorder characterised by inflammation of airway. Allergic asthma is an immunoglobulin E (IgE) related disease. Severe asthma remains difficult to treat. Genetic and genomic approaches of asthma and IgE identified many novel loci underling the disease pathophysiology. Recent epigenetic approaches also revealed the insights of DNA methylation and chromatin modification on histones in asthma and IgE. More than 30 microRNAs have been identified to have regulation roles in asthma. Understanding the pathways of the novel genetic loci and epigenetic elements in asthma and IgE will provide new therapeutic means for clinical management of the disease in future.

Zhang Y. Potential therapeutic targets from genetic and epigenetic approaches for asthma. World J Transl Med 2016; 5(1): 14-25 Available from: URL: http://www.wjgnet.com/2220-6132/full/v5/i1/14.htm DOI: http://dx.doi.org/10.5528/wjtm.v5.i1.14

INTRODUCTION

Asthma runs strongly in families and has a heritability of up to 60%[1]. Allergic asthma, atopic dermatitis and
allergic rhinitis are immunoglobulin E (IgE) related diseases. The Th2 inflammation in airway is a predominant feature of asthma. A sharp increase in the prevalence of asthma was observed in many countries in recent years and a report from the International Study of Asthma and Allergies in Childhood found that the prevalence of symptoms of asthma in children differed more than 20-fold between study centres around the world[2]. Genetic and environmental factors contribute to the prevalence of the disease. The current management of asthma relies on non-specific medication to control airway inflammation and prevent symptoms. Severe asthma remains difficult to treat.

The genetic approaches to asthma include candidate gene studies, positional cloning studies and genome-wide association studies (GWASs)[3]. The gene FCERB on chromosome 11 encoding high-affinity IgE receptor (FɛR) β unit identified almost three decades ago was one of the early mile stones for genetic approaches of asthma[4]. It then turned out the genetic approaches to identify genes underlie complicated diseases were confined by many factors. Genetic associations to asthma for certain locus may be found in one population but may not always be replicated in the other populations. GWAS is powerful approach to overcome the limitations of candidate gene and positional cloning studies. In a GWAS approach the relationship between disease and allele frequencies is examined across a large number of markers spaced in the genome in a big case and control population, robust genetic effects that have substantial population risk can be identified.

Genetic approaches of asthma and IgE have brought remarkable results, but only a small component of the overall genetic contribution to asthma so far has been identified. The missing heritability may be due to rare highly penetrant mutations, multiple small effects, or epigenetic modifications of gene function and other regulating elements for the genome. Epigenetic regulation modifies gene expression that is not caused by changes in the DNA sequence but by DNA methylation, histone modification and other mechanisms. DNA methylation involves the addition of a methyl group to the DNA nucleotide cytosine and adenine which lead to gene silencing. Histones are highly alkaline proteins in eukaryotic cell nuclei that package and order the DNA into nucleosome. The major histone modifications are methylation, acetylation, phosphorylation, ubiquitination and sumoylation. Such modifications affect range from gene activation to gene silencing.

This review discusses the recent discoveries from genetic and epigenetic approaches to asthma and also summarizes the implications of specific loci or regulating elements for therapeutic intervention for asthma.

**Genetic approaches**

More than one hundred genes have been found to have associations with asthma by candidate gene approaches. The candidate gene approach cannot identify novel pathways[5]. Positional cloning is another genetic approach that identifies disease genes by progressive dissection of linkage regions that are consistently co-inherited with the disease. ADAM33[6], PHF11[7], DPP10[8], GPRA[9], HLA-G[10], CYFIP2[11], IRK3[12], OPN3/CHML[13] were discovered as asthma genes by positional cloning. Most associations identified by candidate gene studies and positional cloning studies were moderate. GWAS is more efficient and can be performed to investigate the entire genome simultaneously. It provides the opportunity to identify novel mechanisms of disease pathogenesis.

The first GWAS study for asthma was carried out in the GABRIEL Consortium. The consortium consisted of collaborations among 35 partners across the European Community. In 2007, the consortium reported SNPs in the chromosome 17q12-q21 region to be significantly (P < 10⁻⁶) associated with childhood asthma and asthma associated SNPs were associated with the expression levels of the ORM1-like 3 Saccharomyces cerevisiae (ORMDL3) gene[14]. Then a large consortium GWAS study also confirmed ORMLD3 as an important asthma suspected gene. The consortium also identified IL-18R1, HLA-DRB1, HLA-DQ, IL-33, SMAD3, IL-2RBL, SLC22A5, IL-13 and RORA as asthma or IgE suspected genes[15].

To date, more than ten GWASs on asthma or asthma-relevant traits have been published. Serum YKL-40 levels were shown to elevate in patients with asthma and were correlated with asthma severity, thickening of the subepithelial basement membrane in airway, and pulmonary function[16]. Polymorphisms of Ch13L1 were associated YKL-40 level in 753 Huttenites in a GWAS study for asthma[17]. Polymorphisms of PDE4D, TLE4, ADRA1B, PRNP, DPP10 and GNAI3 were found to associate with asthma in GWASs studies of different populations[18-20]. Polymorphisms of DENND1B and ORMDL3 were also found to associate with asthma in an European American population GWAS study[21]. In another European GWAS study, RAD50, IL-13, HLA-DR-DQ, LRP1B, SNX10, CA10, KCNJ2 were shown associations with asthma[22]. In the EVE Consortium, ORMDL3, IL-1RL1, TSLP, RTP2, IL-33, PYHIN1 were found to associate with asthma[23].

Genome-wide association study identified IL-12A, IL-12RB1, STAT4, and IRF2 genes associated with lung function in asthmatic patients[24]. ORMLD3/GSDMB, IL-1RL1/IL-18R1 loci were also found to associate with severe asthma[25]. In a Danish GWAS study for asthma exacerbations in childhood, GSDMB, IL-33, RAD50 and IL-1RL1 and CDHR3 showed association with asthma[26]. CTNNAL3 and SEMA3D also were associated asthma exacerbation in GWASs studies in two paediatric clinical trials in the United States[27]. IL-4R was found increased in genome-wide expression profiling in allergic asthma[28]. Genome-wide differential gene expression in response to dust mite allergen also identified IL-5, IL-9 and PRG2 to interact with environmental dust mite to increase severe asthma exacerbations in children[29]. In a Japanese GWAS study, TSLP-WDR36 and USP38-GAB1 loci were found to associate with asthma[30]. Lung function, particularly for forced expiratory volume in the first second [FEV(1)] and its ratio to forced vital capacity
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[FEV(1)/FVC], was studied in meta-analyses of GWAS studies. It identified HHH, GPR126, ADAM19, AGER-PPT2, FAM13A, PTC1H, PID1, HTR4, INTS12-GSTCD-NPNT, THSD4 as suspected genes for lung function change[31,32].

**Epigenetic approaches**

Epigenetic effects are other possible causes of asthma. The patterns of gene expression become stably restricted during development, majorly through methylation of CpG sequences and gene silencing. Sex, age, environmental factors and genetic polymorphisms have all been strongly associated with altered methylation at selected loci. To asthma, allergens, microbes, tobacco smoke, diet and metabolism, fish oil, obesity and stress are important environmental factors that influence epigenetic effects in human cells[33]. CD19 (+) B lymphocytes methylation patterns and expression levels showed difference in the locus CYP26A1 in house dust mite allergic patients[34]. Children growing up in a traditional farming environment had lower risk of allergic respiratory diseases. Demethylation of the FOX3 promoter was association with higher number of FOXP3 cells in cord blood mononuclear cells in an extensive farming exposure environment[35]. Hypomethylation of ORMDL1 and STAT6 and hypermethylation of RAD and IL-13 were also found from farm children[36]. DNA methylation in the CD14 promoter was also significantly less in farm mothers[37]. PBMC s from obese asthmatic children had lower levels of promoter methylation of the CCL5, IL-2RA and TBX21 and higher level promoter methylation of TGFBI and FCER2[38]. Recent epigenome-wide approach identified 36 loci that had association with serum IgE level[39]. Among them, DNA methylation events have been found in cytokine signalling genes IL-4, IL-5R, transcription factor genes ZNF22, RAB1, GATA1, KLF1, transmembrane or transporter genes SLC25A33, SLC17A4, SLC43A3, TMEM52B, TMEM41A, ecosinophil associated genes PRG2 and PRG3, phospholipid metabolism genes LPCAT2, CLC and MEMB68, and metabolic enzyme genes L2HGDH, CEL, KEL, PDE6H, EFNA3, ALDH3B2.

Noncoding RNAs emerged as novel molecules that are important in lung diseases in recent years[40]. Noncoding RNAs include housekeeping RNAs, long noncoding RNAs and small noncoding RNAs. Micro RNAs (miRNAs) are the most studied small noncoding RNAs. miRNAs are about 18-25 nucleotide long noncoding RNAs that silence target mRNA. More than 3000 human miRNA genes have been identified so far. There is a significant number of miRNAs that are still uncharacterized[39]. miRNAs induce messenger RNA (mRNA) degradation and then inhibit the translation. miRNAs can target 60% of mRNAs and control the signal pathways in most cell types[41]. More than 30 miRNAs have been found to associate with asthma[42]. These miRNAs regulate epithelium cells, airway smooth muscle cells and T2 response.

To date, it is not reality to assume that genetic targets and regulating elements for asthma identified by genetic and epigenetic approaches can be accessed either by biologics (antibodies and proteins) or small molecules (drugs), but several genes regulate in pathways from epithelial damage to the adaptive immune system in asthma, providing a new means for effective therapies. This review focuses on the novel genes expressing on human airway epithelium cells and cytokine networks that play important roles in asthma pathophysiology. It also summarizes the miRNAs that were found to regulating asthma pathogenesis.

**THE POTENTIAL THERAPEUTIC TARGETS FOR ASTHMA IN EPITHELIAL CELLS**

Human airway epithelium is now believed to be central to the pathogenesis of asthma[43,44]. Several asthma candidate genes identified by genetic and epigenetic approaches may modify the inflammatory response to epithelial damage or regulate homeostatic and healing pathways. The following novel genes identified by GWASs express in the airway epithelium and understanding their pathways in inflammation response will provide unique opportunities to develop new therapeutic means for asthma (Table 1).

**ORMDL3**

The association signals on human chromosome 17 with asthma are maximal within an island of linkage disequilibrium that contains ORMDL3, GSDMA and GSDMB. Now the associations have been found in many GWAS studies. The loci were not only associated childhood asthma, but also associated with severe asthma or asthma exacerbations. ORMDL3 protein is found in the membranes of the endoplasmic reticulum (ER). ER stress is one of important stage linked to cellular responses to inflammation[45]. ORMDL3 has been found to be up-regulated in transcriptional activator XBP-1(S)[46]. ORM gene expression regulates sphingolipid metabolism[47]. Ceramide and sphingosine-1-phosphate (S1P) are two important bioactive signalling sphingolipids. They mediate cell survival, proliferation, apoptosis, differentiation and cell-cycle arrest[48]. Clinical observation showed that they were increased in asthmatic airways[49]. Recent study showed Ormdl3 may regulate ceramide level in epithelial cells and then regulate the inflammation response[50]. Transfection of ORMDL3 in human bronchial epithelial cells in vitro induced expression of many chemokines and selectively activated activating transcription factor 6, suggest an ER UPR pathway through which ORMDL3 may be linked to asthma[51]. ORMDL3 also regulates eicosphin trafficking, recruitment and degranulation[52]. ORMDL3 was shown to modify SERCA in the ER and induce inflammation[53]. A recent study showed in 17q21 risk allele carrier children their mononuclear cells significantly increased IL-17 secretion[54]. ORMDL3 may influence multiple pathways in the ER that mediate inflammation during asthma and regulating ORMDL3 may have the potential therapeutic effects on inflammation disease such as asthma.
### GSDMB and GSDMA

The human chromosome 17 locus of asthma covers a genomic area of approximately 200Kb. ORMDL3 and GSDMB reside in one island of linkage disequilibrium that contains all the maximally associated SNPs. Independent associations are also detectable telomERICally near the GSDMA which may make contributions to asthma susceptibility as well\(^\text{[14]}\). The GSDM family genes were first identified in mouse. They are expressed primarily in the gastrointestinal tract and expressed a lower level in the skin. The mouse syntenic homology areas including mouse Gsdm1, Gsdm2 and Gsdm3 are on mouse chromosome 11. Mouse Gsdm proteins contain DFN15 domain of Pfam domains. They are expressed predominantly in the gastrointestinal tract and in the skin\(^\text{[58]}\), in a highly tissue-specific manner\(^\text{[58]}\). In humans GSDMA and GSDMB are expressed in the gastrointestinal and bronchial epithelium. Members of the gene family may have a role in regulation of apoptosis\(^\text{[57]}\). GSDMA was shown to mediate cell-growth inhibition. GSDMB is expressed in stem cell-resided region and has a potential role in stem cell proliferation. The GSDMB-driven HSVtk expression vector had a therapeutic effect on the occult peritoneal dissemination (PD) model mice. This strategy can potentially be used to treat GC patients with PD in clinical\(^\text{[58]}\). The specific expression of GSDMB and GSDMA in epithelium may also service to therapeutic means to asthma in future.

### Thymic stromal lymphopoietin

Thymic stromal lymphopoietin (TSLP gene) was found to associate with asthma by GWAS and SNPs in TSLP may have asthma risk through up-regulating its mRNA expression or the protein secretion\(^\text{[59]}\). It expresses mainly by epithelial cells at barrier surfaces (skin, gut and lung)\(^\text{[60,61]}\). TSLP plays a critical role in orchestrating the inflammatory response and a critical factor in airway remodelling in asthma. Airway remodelling is a repair process that happens after injury resulting in airway hyper-responsiveness in asthma. TSLP induces cellular senescence during airway remodelling in asthma\(^\text{[62,63]}\). Myeloid dendritic cells (DCs) are the cell populations with the highest known co-expression of the TSLP receptor and its associated subunit IL-7R. Treatment of human DCs with TSLP induces improved survival, up-regulation of major histocompatibility complex class II and the production of a variety of chemokines\(^\text{[60]}\). It promotes Th2 cytokine-associate inflammation by directly promoting the effector functions of CD4\(^+\) Th2 cells\(^\text{[61]}\).

### SMAD3

SMAD3 encodes SMAD (mothers against decapentaplegic homolog) family member 3 and has a role in modifying tumour growth\(^\text{[64,65]}\) through the transforming growth factor-beta (TGFβ) pathway\(^\text{[66]}\). SMAD3 is concentrated in the nuclei of bronchial epithelial cells and macrophages and functions as a transcriptional modulator activated by TGFβ. The family members of TGFβ maintain immune function in lung\(^\text{[67]}\) and the TGFβ signalling pathways can be activated after allergen challenge in mice\(^\text{[68]}\). A mouse knockout of Smad3 showed accelerated wound healing and an impaired local inflammatory response\(^\text{[69]}\), even though mice lacking Smad3 may exhibit increased baseline levels of pro-inflammatory cytokines in their lungs\(^\text{[70]}\). Smad3 signalling is required for myogenic differentiation of myoblasts\(^\text{[71]}\), this may be linked a role in airway smooth muscle hypertrophy.

### DPP10

DPP10 was the only gene that was identified both by positional cloning and GWAS studies. DPP10 genetic variants could affect lung function decline in aging and also associate aspirin-exacerbated respiratory disease. The DPP proteins have a β-propeller that regulates substrate access to an α/β hydrolase catalytic domain. Unlike other DPP family members, DPP10 lack of enzymatic activity is unable to cleave terminal dipeptides from asthma-related cytokines and chemokines\(^\text{[8]}\). In neurons, DPP10 forms part of the A-type K+ (Kv4) ion channel complex and DPP10 variants accelerate channel gating kinetics. It is not clear what exact roles of DPP10 in the airway epithelial cells, the future research will focus on how DPP10 regulate inflammation response in epithelial cells in asthma by applying animal models and cellular models.

### Cadherin-related family member 3

Cadherin-related family member 3 (CDHR3) is a transmembrane protein with six extracellular cadherin
domains. The biological function of CDHR3 remains. It belongs to the cadherin family of transmembrane proteins that have function roles in homologous cell adhesion. It is important for epithelial polarity, cell-cell interaction and differentiation[72]. Other members including E-cadherin of the family have been associated with asthma[73]. CDHR3 Protein structure modelling showed that the CysS29Tyr risk-associated alteration was located at the interface between two D5 and D6 membrane-proximal cadherin domains. The variant residue may interfere with interdomain stabilization, folding or conformation[26].

**Semaphorin-3D**

Semaphorin-3D (SEMA3D) is a member of the semaphorin class 3 signalling molecules. SEMA3A and SEMA3E are secreted transmembrane proteins involved in immune response and the recruitment of CD4+ and CD8+ T cells[74]. SEMA3D is responsible for endothelial cell migration[75] and has been shown to be essential for healthy angiogenesis during development[51]. Angiogenesis is also a feature of airway remodelling. It is possible that SEMA3D plays a role in airway remodelling from plausible mechanisms. It directs angiogenesis and airway epithelium migration, resulting in a reduction of epithelial cells. Like other semaphorins, it has effects on immune cell recruitment during the inflammatory response, which leads to remodelling[27].

### Table 2 The genetic and epigenetic loci modify cytokines and receptors of asthma

| Genes | Chromosome location | Phenotypes methods | Identifying and functions in asthma | Possible pathways | Ref. |
|-------|---------------------|--------------------|-------------------------------------|------------------|-----|
| IL-18R1 | 2 | Asthma | GWAS | Activation of NF-κB, inducing T1-associated cytokines | [15,25] |
| IL-1RL1 | 2 | Asthma, Eos | GWAS | Receptor for IL-33 | [15,23,94] |
| IL-5RA | 3 | IgE | Epigenetics | Tr1 inflammation, regulating eosinophils | [39] |
| IL-12A | 3 | Lung function | GWAS | Tr1 regulation, activating IFN-γ | [24] |
| IL-4 | 5 | IgE | Epigenetics | Tr2 inflammation, promoting IgE class switching | [39] |
| IL-13 | 5 | Asthma, IgE | GWAS/epigenetics | Tr2 inflammation, promoting IgE class switching | [15,22] |
| IL-5 | 5 | Asthma | GWAS/epigenetics | Tr2 inflammation, regulating eosinophils | [15,22] |
| IL-9 | 5 | Asthma | Expression profiling | Stimulates cell proliferation and prevents apoptosis | [29] |
| IL-33 | 9 | Asthma | GWAS | Inducing T1-associated cytokines | [15,23,26,94] |
| IL-2RA | 10 | Asthma | Epigenetics | PI3K-Akt signalling pathway and Akt signalling | [38] |
| IL-4R | 16 | Asthma | Expression profiling | Tr2 inflammation | [28] |
| IL-12RB1 | 19 | Lung function | GWAS | Tr1 regulation, activating IFN-γ | [24] |
| IL-2RB | 22 | Asthma | GWAS | Endocytosis and transducer mitogenic signals | [15] |
| | | | | | |

GWAS: Genome-wide association study; IL: Interleukin; IgE: Immunoglobulin E; IFN-γ: Interferon-γ; NF-κB: Nuclear factor kappa-B.

**THE POTENTIAL THERAPEUTIC TARGETS IN CYTOKINE NETWORKS FOR ASTHMA**

Genetic and epigenetic approaches of asthma and IgE have revealed many cytokines and cytokine receptors that regulate the inflammation in the airways. These cytokines and cytokine networks play critical roles for inflammation response in epithelium cells and immune cells. Specific targeting the cytokines and the networks may provide new therapeutic means to asthma. The cytokines identified by GWAS and epigenetic approaches are discussed here (Table 2).

**IL-33, IL-18R1 and IL-1RL1**

IL-33, IL-18 and IL-1 belong to the IL-1 family of cytokines that alter host responses to inflammatory and infectious challenges. They employ their functions through a toll-like receptor-IL-1 receptor (TLR-IL-1R) superfamily. IL-1 receptor signalling activates transcription factor nuclear factor kappa-B (NF-κB), mitogen-activated protein (MAP) kinases p38, JNK, and ERK1/2[27].

IL-33 was originally identified as a nuclear factor in vascular endothelial cells[27], and was subsequently detected in airway epithelial cells[39,80]. The activities of IL-33 as a nuclear factor remain unclear[24]. IL-33 is constitutively expressed and has function as an endogenous danger signal to alert the immune system after endothelial or epithelial cell damage during trauma or infection stresses[81]. A mouse IL-33 gene knockout has shown IL-33 works as a crucial amplifier of innate immunity[83]. IL-33 expression is induced by a range of environmental and endogenous triggers, suggesting an essential role during infection, inflammation and tissue damage[84]. IL-33 activates a heterodimeric receptor complex containing IL-1RL1 (ST2) and IL-1 receptor accessory protein (IL-1RAP), leading to activation of NF-κB and MAP kinases and then drives production of Tr2 cytokines IL-4, IL-5, and IL-13[29].

The IL-18R1 gene is located on chromosome 2q. It form a gene cluster along with four other members of the interleukin 1 receptor family [IL-1R2, IL-1R1, IL-1R2 (IL-1Rrp2), and IL-1RL1 (TI/ST2)] on the loci. IL-18R1 and IL-1RL1 flank each other with the same
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orientation of translation. They are within the same island of linkage disequilibrium and it has not yet been possible to assign the genetic effects at this locus to one gene or the other. It is possible that both genes may be co-regulated. IL-1RL1 encodes the receptor of IL-33. IL-18 is closely related to IL-33[29] and synergizes with IL-12 to induce interferon gamma and to promote Th1 responses[85]. These loci therefore identify a pathway for the communication of epithelial damage to the adaptive immune system and a potential switch point for choosing between Th1 or Th2 responses.

IL-2RB
IL-2RB encodes the beta receptor of IL-2. IL-2 is secreted by antigen-activated T cells. It controls the survival and proliferation of regulatory T cells[86] and plays a prominent role in the maintenance of natural immunologic self-tolerance[87]. The IL-2 receptor has α (CD25), β (CD122) and γ chains[88]. The β chain (IL-2RB) is a signal transduction element that is also present in the IL-15 receptor. It belongs to the type I cytokine receptor family and has no intrinsic kinase activity[89]. The receptor regulates T cell-mediated immune responses through endocytosis, whereby ectodomain shedding of IL-2Rβγ generates an intracellular fragment[89]. In a mouse model of asthma, local inhibition of IL2rb restored an immunosuppressive cytokine milieu that ameliorated lung inflammation[90].

IL-4 and IL-4R
IL-4 is adjacent to RAD50 on chromosome 5. The locus is exceptional in showing strong association to IgE in addition to doctor-diagnosed asthma[15]. The 3’ end of RAD50 has several enhancer elements and conserved non-coding sequences that act as a locus control region for IL-4 and IL-13[81]. IL-4 is one of the key Th2 cytokines and immunoglobulin class switching in B cells. IL-4 methylation was associated with IgE production[89]. IL-4R is the best candidate allergic biomarker and shows to have association with allergic asthma in a genome-wide expression profiling study[28]. A soluble form of the IL-4 receptor can block B cell-binding of IL-4 or other IL-4R antagonists[92].

IL-5 and IL-5RA
IL-5 encodes a growth and differentiation factor for B cells. IL-5 also controls the activation and localization of eosinophils[93]. A SNP (rs4143832) located near IL-5 on 5q31 showed to have association with blood eosinophil counts[34]. Eosinophils are an important source of cytokines and chemokines at the allergic inflammation sites[93]. IL-5RA was methylation different with asthma[29]. IL-5RA encodes a receptor that selectively stimulates eosinophil production and activation[96]. In clinic, therapies directed at eosinophil may be effect in a subgroup of refractory asthma individuals[97].

IL-13
IL-13 encodes an immunoregulatory cytokine primarily by activated Th2 cells. IL-13 is involved in several stages of B-cell maturation and differentiation. It up-regulates CD23 and MHC class II expression. It also promotes IgE isotype switching of B cells. IL-13 down-regulates macrophage activity and inhibits the production of pro-inflammatory cytokines and chemokines. This cytokine is critical to the pathogenesis of allergen-induced asthma but works through mechanisms independent of IgE and eosinophils. rs20541 (Arg130Gln or IL13 +4257GA) in the coding region of IL-13 has been shown to be associated with asthma[98] and total serum IgE levels[99]. One GWAS study confirmed the important role of Th2 cytokine and antigen presentation genes in asthma[95].

IL-12A and IL-12RB1
IL-12 is a key cytokine that regulates innate and adaptive immune responses. IL-12 is composed of the p35 subunit and the p40 subunit (encoded by IL-12A and by IL-12B respectively). The formation of the high-affinity IL-12 is lead by the co-expression and dimerization of the IL-12RB1 and IL-12RB2 proteins. IL-12 activates interferon-γ (IFN-γ) production. STAT4 regulates the response of lymphocytes to IL-12; it induces the expression of IL-12RB2 and transcription factor IRF1. IRF1 is induced by IFN-α, IFN-β, and IFN-γ. IRF2 can competitively inhibit the expression of genes induced by IRF1. The IL-12-STAT4-IFN-γ signalling pathway is essential for the differentiation of naive Th0 cells into Th1 cells[24].

IL-9
IL-9 was found to interact with environmental dust mite to increase severe asthma exacerbations in children[29]. IL-9 induces cell proliferation and prevents apoptosis through the IL-9R. IL-9R activates different STAT proteins. IL-9 has been shown to promote mast cell recruitment to the lung, increase mast cell activity, and enhance airway remodelling in a murine model of asthma and also mast cells act as the main expressers of IL-9 receptor in human asthmatic lung tissue[100]. IL-9 production from bronchoalveolar lavage lymphocytes increases after an inhaled allergen challenge in atopic asthmatic patients[101] and IL-9 has been shown to up-regulate expression of eotaxin in cultured human airway smooth muscle cells[102].

miRNAs AND THEIR REGULATIONS IN ASTHMA

miRNA can act as a regulator between genetic and environmental factors in the pathogenesis of asthma. Epigenetic changes are potentially reversible and therapeutic modulation of miRNAs may provide opportunities to regulate or suppress allergic inflammation[103]. There are more than 11 miRNAs differentially expressed in human exhaled breath condensate from asthma patients compared with health subjects[104].
570-3p was found to have lower level in serum and exhaled breath condensate from asthma patient[105]. miR-221, miR-146a and miRNA146b has been found to have altered expressions in asthmatic patients airway smooth muscle[42,106]. There are number of miRNAs down-regulated or up-regulated in nasal biopsies of asthma patients[107]. Here the most potential miRNAs that could be used as therapeutic targets for asthma are discussed (Table 3).

miR-1
Vascular endothelial growth factor (VEGF) is an important regulator of pulmonary T\(\text{h}2\) inflammation. Lung-specific overexpression of VEGF can decrease miR-1 expression in the endothelium of lung. Intranasal delivery of miR-1 inhibited inflammatory responses to allergen ovalbumin, house dust mite, and IL-13 overexpression. Myeloproliferative leukaemia (Mpl protein) is the receptor for thrombopoietin and has roles in megakaryopoiesis and hematopoietic stem cell differentiation[108]. VEGF controlled the expression of endothelial Mpl during T\(\text{h}2\) inflammation via the regulation of miR-1. In vivo silence of Mpl inhibited T\(\text{h}2\) inflammation. It indirectly inhibited the expression of P-selectin in lung endothelium. These experiments defined a novel VEGF-miR-1-Mpl-P-selectin effector pathway in lung T\(\text{h}2\) inflammation. The utility of miR-1 and Mpl may be potential therapeutic targets for asthma management[109].

miR-126a
In a mouse model, blockage of miR-126 suppressed the asthma phenotype, resulting in diminished T\(\text{h}2\) response, inflammation, airway hyper-responsiveness, eosinophil recruitment and mucus over secretion. In vivo activation of TLR4 by house dust mite antigens led to the induction of allergic disease, a process that is associated with expression of many small, noncoding miRNAs. miR-126 inhibition resulted in augmented expression of POU domain class 2 associating factor 1 that regulated GATA3 expression. Targeting miRNA-126a in the airways may lead to anti-inflammatory treatments for allergic asthma[110].

miR-221
The mass of airway smooth muscle (ASM) is increased as a feature of asthmatic airways. Increased miR-221 expression was found in ASM cells from individuals with severe asthma. miR-221 increased ASM proliferation and IL-6 release. In severe asthma patients the inhibition of miR-221 reduced proliferation and IL-6 release. miR-221 regulated p21(WAF1) and p27(kip1) expression levels and regulated the hyper-proliferation and IL-6 release of ASM cells from severe asthma patients[42].

miR-146a and miR-146b
miR-146a and miR-146b gene expressions were a pattern of induction in response to a variety of microbial components and pro-inflammatory cytokines. miR-146a is a NF-\(\kappa\)B dependent gene. miR-146a/b were predicted to base-pair with sequences in the 3′UTRs of the tumor necrosis factor (TNF) receptor-associated factor 6 gene and IL-1 receptor-associated kinase 1 gene. These genes encode two key adapter molecules of Toll-like and cytokine receptors. miR-146 controls toll-like receptor and cytokine signalling. It works through a negative feedback regulation loop involving down-regulation of IL-1 receptor-associated kinase 1 and TNF receptor-associated factor 6 protein levels[111].

miR-150
miR-150 down-regulated transcription factor c-Myb that regulates lymphocyte development. MiR-150 is specifically expressed in mature lymphocytes. c-Myb is a transcription factor controlling lymphocyte development. In vivo miR-150 controls c-Myb expression in a dose-dependent manner over a narrow range of miRNA and c-Myb concentrations. MiR-150 and other miRNAs have evolved to control the expression of a few critical target proteins in particular cellular contexts[112]. c-Myb is an important regulator of Gata3[113]. c-Myb and GATA-3 cooperatively regulate IL-13 expression as regulate IL-13 expression[114].

miR-155
Like miR-146a, miR-155 is one of the most frequently studied miRNAs in both innate and adaptive immune response. Mice without miR-155 displayed increased airway remodelling and were unable to produce the cytokines for immune system homeostasis and function[115,116]. miR-155 targets transcription factor c-Maf, which promotes T\(\text{h}2\) cells to generate IL-4, IL-5 and

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Table 3  The microRNAs and their potential roles in asthma

| miRNA     | Possible function roles in asthma                                                                 | Ref.          |
|-----------|--------------------------------------------------------------------------------------------------|---------------|
| miR-1     | Targeting Mpl to regulate T\(\text{h}2\) inflammation and P-selectin in lung endothelium          | [109]         |
| miR-126a  | Regulating T\(\text{h}2\) inflammation, airway hyper-responsiveness, eosinophil recruitment     | [110]         |
| miR-221   | Mediator IL-6 proliferation in airway smooth muscle                                              | [42]          |
| miR-146a  | NF-\(\kappa\)B dependent gene, control toll-like receptors and cytokine signalling                | [111]         |
| miR-146b  | NF-\(\kappa\)B dependent gene, control toll-like receptors and cytokine signalling                | [111]         |
| miR-150   | Down-regulated transcription factor c-Myb to control lymphocyte development                     | [112]         |
| miR-155   | Targeting c-Maf to promote T\(\text{h}2\) cells to generate IL-4, IL-5 and IL-10                  | [115,116]     |

IL: Interleukin; NF-\(\kappa\)B: Nuclear factor kappa-B.
IL-10 cytokines.

**FUTURE RESEARCH DIRECTIONS**

The genetic and epigenetic approaches identified many novel loci and regulating elements in human genome. The airway epithelial expressions of some loci and inflammatory cytokines in asthma provide unique therapeutic targets. Regulating elements such as miRNAs also can be served as potential therapeutic targets for the disease. RNA sequencing, deep DNA sequencing, ChIP-sequencing, exome sequencing, transcript profiling and miRNA profiling are becoming more and more powerful platforms to discover more genetic variants, regulators of transcriptions that are more and more powerful platforms to discover more genetic models, animal models and pharmacological models for these novel loci and regulation elements will eventually decipher the precise functions of these targets and it will provide new therapeutic means for asthma in future.

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