

# New Insights Into the Relationship Between Chitinase-3-Like-1 and Asthma

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## Abstract

**Purpose of Review** CHI3L1 (also known as YKL-40), a member of “mammalian chitinase-like proteins,” is a serum protein lacking enzymatic activity. Although the protein is highly conserved in mammals, a consensus regarding its role in human pathologies is currently lacking. In an attempt to shed light on the many physiological functions of the protein, specifically with regard to asthma, a comprehensive overview of recent studies is provided.

**Recent Findings** In asthma, CHI3L1 is secreted from macrophages and airway epithelial cells through an IL-13 related mechanism. Th2-associated inflammatory responses due to allergen exposure, resulting in airway hyper-responsiveness and smooth muscle contraction, play a role in tissue remodeling.

**Summary** The importance of CHI3L1 in initiation and development of asthma is not limited to its involvement in highly orchestrated events of inflammatory cytokines but further research is needed for further elucidation. Levels of the protein are associated with severity for numerous pathologies, including asthma, suggesting limited specificity as a biomarker.

**Keywords** CHI3L1 · YKL-40 · Chitinase · Chitinase-like protein · CD4+Th2 lymphocytes · IL-4 · IL-13 · Asthma · Biomarker · SNP

## Introduction

CHI3L1 (chitinase-3-like-1 protein), also known as YKL-40, is a human glycoprotein that binds to chitin, but does not possess chitin hydrolase activity. It is secreted by a variety of cells, including neutrophils [1] and macrophages [2] and expression is induced by IFN- $\gamma$  [3] and release stimulated by IL-6 [4]. CHI3L1 levels have been also associated with pathologic conditions such as asthma, atopy, and other immune-related phenotypes [5–7]. Circulating CHI3L1 levels are correlated positively with asthma severity, thickening of the lung subepithelial basement membrane, frequency of rescue inhaler use, and deterioration in pulmonary function in asthmatic subjects studied [8]. The activation of type 2 helper (Th2) lymphocytes leads to secretion of cytokines such as IL-13 and orchestrates further events in pathogenesis of asthma [9]. IL-13 secretion results in the alternative activation of alveolar macrophages, and these activated macrophages have been reported to play a role in the propagation of airway inflammation [10]. Respiratory epithelial cells, alveolar macrophages, and neutrophils are known to secrete CHI3L1 in human airways, and hence, it has been attributed a role in airway inflammation [8] (Fig. 1).

Furthermore, animal model studies have shown that this molecule has an outstanding role in various steps of asthma pathogenesis by increasing allergic sensitization, propagating Th2 inflammation, and regulating inflammatory cell apoptosis [11]. The contribution of CHI3L1 in different aspects of asthma such as initiation, development, and also its control is dominated by the role of released cytokines and genetic

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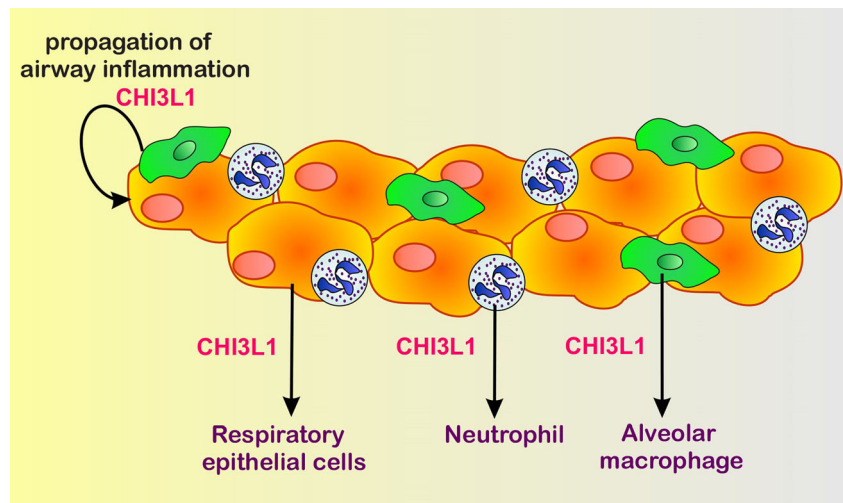
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**Fig. 1** CHI3L1 is expressed in a number of human cells independent of their origin such as mesenchymal, epithelial, or hematopoietic. In asthma, it has been known to contribute to tissue remodeling. Immunostaining of bronchoalveolar-lavage specimens from patients with severe asthma revealed the main cells producing CHI3L1 in airways



factors. There are many SNPs related to asthma reported in CHI3L1 encoding gene located on chromosome 1q32.1 [12•].

### General Properties

The CHI3L1 protein as a minor whey glycoprotein primarily was found in secretions of bovine mammary glands during non-lactating periods. Surprisingly, the discovered protein was not found in milk or colostrum, was not formally named (except as a 39-kD glycoprotein), and was postulated to be a marker of mammary function during non-lactating periods. Using heparin-agarose affinity chromatography it was partially purified [13, 14] and it took almost a decade to be officially confirmed as CHI3L1 [15]. The protein was subsequently identified as a secreted product of human synovial cells [16] and MG-63 osteosarcoma cells [17]. In the latter report, the protein was named YKL-40 in which three capital letters were amino acid symbols for the first three N-terminal amino acids—tyrosine (Y), lysine (K), and leucine (L)—and the molecular weight as inferred from SDS-PAGE [18–20]. CHI3L1 appears in the literature under quite different names due to the discovery by a number of researchers (Table 1). The gene encoding CHI3L1 in human is localized in a highly conserved area on chromosome 1q32.1 [26] and has been isolated in 1997 [25, 27]. It contains a single polypeptide chain of 383 amino acids and has an isoelectric point of 7.6 [28]. CHI3L1 gene with a size of 7948 base pairs consists of 10 exons [25, 27]. Two splice forms of this gene are reported; isoform 1 contains exons 1–10 whereas isoform 2 possesses spliced exon 8 [29]. Structurally, CHI3L1 is a glycoprotein belonging to “Family 18 chitolectins” [30–32] and “Mammalian chitinase-like proteins.” It is a highly conserved protein [33] that has been reported in various organisms in addition to human [34–39] (Fig. 2).

Although the molecule has no catalytic activity it has been given the designation E.C.3.2.1.14. Enzymatically

active chitinases share a conserved active site sequence motif, DXXDXDXE, in which the terminal glutamate is a key catalytic residue. In CHI3L1, the key glutamate (aa140) has been replaced by leucine. Moreover, the preceding aspartate (aa 138), participating in catalysis, is replaced by alanine. The X-ray crystallographic findings revealed structural configuration of CHI3L1 as a (beta/alpha) 8-barrel, fold with an inserted alpha + beta domain, same as other chitinase family members. A 43-Å long carbohydrate-binding cleft is also placed at the C-terminal side of the beta strands in the (beta/alpha) 8-barrel [40, 41].

Electrostatic interactions between the negatively charged heparin and the positively charged CHI3L1 make it possible to apply heparin columns successfully in purification of CHI3L1 [40]. Activated macrophages, neutrophils, activated chondrocytes, synoviocytes, and smooth muscle cells are considered as main sources of CHI3L1 in non-transformed tissues [22, 24, 25], while it is not expressed by monocytes and does not appear until the later differentiation stages of macrophage [42]. The monocytes maturation to macrophages is induced by this glycoprotein [43–46]. The expression is induced by IFN- $\gamma$  [3] and release stimulated by IL-6 [4]. Immunohistochemical studies revealed that cells with higher metabolic activity and/or proliferation have especially high YKL-40 expression [29, 47]. In spite of not having catalytic activity, CHI3L1 binds with significant affinity to chitin and with lower affinity to heparin and type I collagen [20, 22, 23, 25–28, 48], in which the latter represents the first documented interaction between CHI3L1 and another protein [20] (Fig. 3).

Interestingly, CHI3L1 has been reported to be a growth, adhesion, and chemotactic factor for fibroblasts, eosinophils, vascular smooth muscle cells, chondrocytes, and synovial cells [23, 35, 49–51]. CHI3L1 induces the

**Table 1** Multiple names of chitinase-3-like-1

| Name                                        | Discovery                                                                                  | Reference |
|---------------------------------------------|--------------------------------------------------------------------------------------------|-----------|
| YKL-40                                      | Major secreted protein of human MG-63 osteoblast cells                                     | [17]      |
| HC-gp39 (human cartilage glycoprotein 39)   | Major secreted protein of articular chondrocytes and synovial cells                        | [21, 22]  |
| gp38k (38-kDa heparin-binding glycoprotein) | 38-kD heparin-binding glycoprotein in differentiating vascular smooth muscle cell cultures | [19, 23]  |
| Chondrex                                    | Chondrocyte protein upregulated in RA patients                                             | [24]      |
| CHI3L1 (chitinase-3-like-1)                 | Approved human genome project gene name; abbreviation for chitinase 3-like-1               | [25]      |

proliferation of chondrocytes and synovial cells [35] and stimulates the growth of several types of human fibroblast derived from the fetal lung, adult skin, and synovium [52]. It also acts as chemoattractant for endothelial cells, modulating vascular endothelial cell morphology by promoting the formation of branching tubules [50]. Thus, CHI3L1 plays a role in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells. Collectively, these data suggest involvement of CHI3L1 in the pathologic process of human diseases with tissue remodeling [53]. Significant CHI3L1 protein expression is reported in morphogenesis by relevant cell populations during early human development [29]. As a normal component of human serum, CHI3L1 is present with a mean value of 80  $\mu\text{g/l}$  in healthy children, and gradually increases to 102  $\mu\text{g/l}$  by adulthood, with no significant differences between males and females [54]. The detailed functions of CHI3L1 have not yet been clarified, but earlier studies suggested its involvement in angiogenesis [19, 50, 55]. This glycoprotein may contribute to tissue

remodeling and extracellular matrix (ECM) degradation [56]. Blockade of CHI3L1 activity or expression in xenografted SCID mice decreases tumor growth, angiogenesis, and metastasis [57, 58].

Elias et al. reported that chitinase-3-like 1 regulates cellular and tissue responses via IL-13 receptor  $\alpha 2$ . Using wild type mice, IL-13R $\alpha 2$  null ( $-/-$ ) mice, and Chi3l1/YKL-40 transgenic mice (Tg+), they successfully showed that melanoma cell administration causes impressive levels of metastasis in lungs from wild type mice. The metastatic response was markedly increased in Tg+ mice in which YKL-40 was selectively targeted to the lung [59••] (Fig. 4).

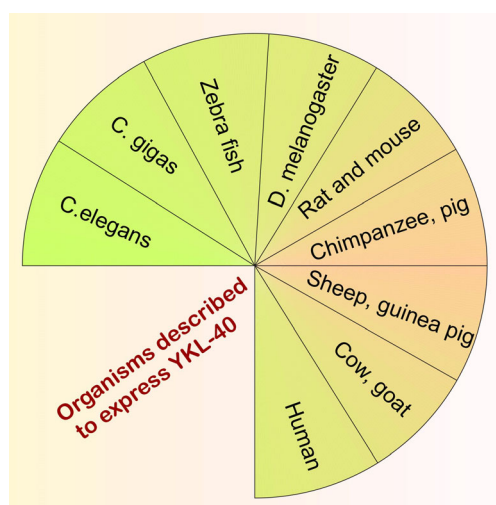


**Fig. 2** CHI3L1 has been reported in many organisms in spite of lacking chitin metabolism. CHI3L1 in human for example plays a pivotal role in regulation of cell proliferation, adhesion, migration, and activation

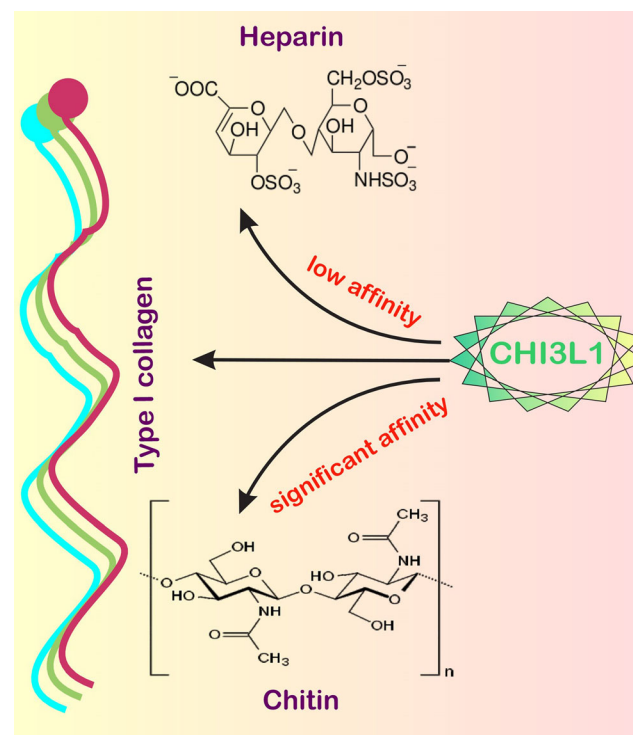


**Fig. 3** CHI3L1 binds with different affinity to several structures. It binds heparin, suggesting that it could interact with heparan sulfate proteoglycans in vivo. CHI3L1-binding ability to collagen was also assessed by affinity chromatography

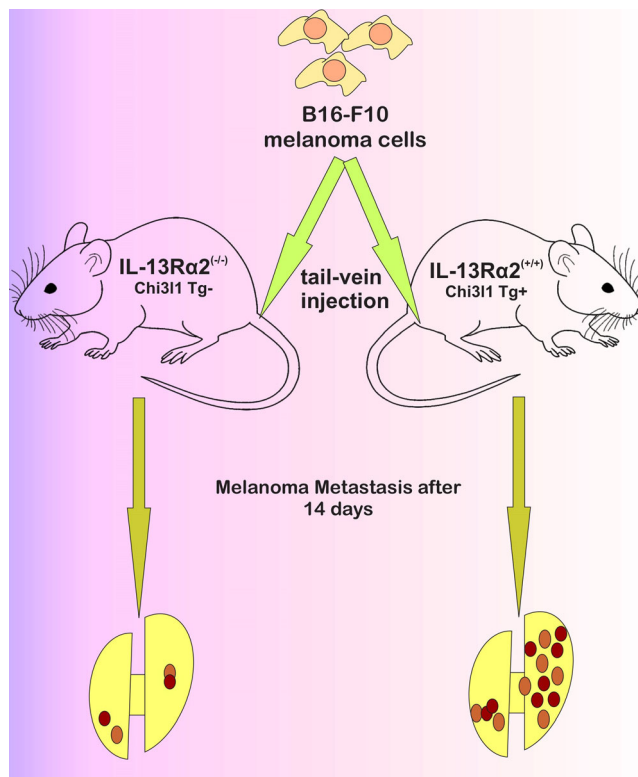


**Fig. 4** In vivo interaction between Chi3l1 and IL-13R $\alpha$ 2 in melanoma metastasis was shown by tail-vein injection of B16-F10 melanoma cells to WT mice, IL-13R $\alpha$ 2 null ( $-/-$ ) mice, Chi3l1/YKL-40 Tg mice (Tg+), and Chi3l1/YKL-40 Tg/IL-13R $\alpha$ 2 null mice. Fourteen days after injection, melanoma metastasis was visually assessed (only IL-13R $\alpha$ 2 null ( $-/-$ )/Chi3l1 Tg- and IL-13R $\alpha$ 2 ( $+/+$ )/Chi3l1 Tg+ have been demonstrated and other genotypes show an intermediate pattern)

### CHI3L1: a Biomarker in the Field of Translational Medicine

CHI3L1 is expressed in many types of cancer cells, and elevated plasma levels are predictive of poor prognosis in patients with different types of cancer. Patients with metastatic disease, short recurrence/progression-free intervals, and short overall survival show the highest plasma CHI3L1 levels [26, 60–65]. High CHI3L1 protein expression has also been found in carcinoma cells from the breast [60–62], colon [26], liver [63], cervix [26], head/neck [64], melanoma [26], as well as within tumor-associated macrophages, mast cells, and leukocytes [1, 47, 62, 64, 65]. In patients suffering myocardial infarction (MI), higher YKL-levels have been documented [66–68]. Surprisingly, CHI3L1 seems to be a useful prognosticator (indicator of prognosis) and also a potential “tumor marker” in screening and monitoring of cancer patients [69–72]. CHI3L1 is applicable not only in cancer diagnosis but also in a wide range of pathological conditions. Barkay et al. showed its expression is induced in the CNS of human and non-human primates with lentiviral encephalitis [73]. Dong Wang et al. reported overexpression of CHI3L1 in the

spleen of patients with portal hypertension [74]. CHI3L1 level is also elevated both in patients with type 1 and type 2 diabetes. Kwon et al. found that its serum level is correlated with symptom severity in patients with allergic rhinitis [75].

### Association Between CHI3L1 and Asthma

Asthma is one of the most common chronic diseases in the world, affecting people of all ages and ethnic backgrounds. Nearly 300 million people around the world suffer from asthma, and it is projected that this number will increase to 400 million by year 2025 as more countries become urbanized [76, 77]. The disease in general is not a life-threatening but mortality rates associated with it are considerable, accounting for about 1 in every 250 deaths worldwide [78]. It is characterized by airway inflammation, mucus hypersecretion, airway hyper-responsiveness (AHR), and infiltration of eosinophils into the bronchial wall and lumen [79, 80, 81]. Subepithelial fibrosis develops due to accumulation of collagen fibers and proteoglycans under basal membrane in asthma [82, 83]. Such changes may affect asthma severity, and end up in relative irreversible obstruction of airways [84] (Fig. 5).

Histologically, highly variable damage and shedding of the airway surface epithelium are reported in asthma: some airways have intact surface epithelium even in the presence of marked inflammation and other structural changes [86]. Loss of epithelium induces a healing process that results in either complete restoration of the columnar/cuboidal epithelium with normal proportions of goblet and ciliated cells or, if the injury is repeated, squamous cell metaplasia and/or goblet cell hyperplasia [87–89].

The onset and progression of allergic asthma is accompanied by a complex series of overlapping and concurrent inflammatory responses, orchestrated by CD4<sup>+</sup> Th2 lymphocytes [90]. Th2 lymphocytes produce a characteristic array of cytokines that are implicated in the initiation and perpetuation of airway inflammation. Interleukins IL-4 and IL-13 stimulate B cell activation and class switching to immunoglobulin E. The released IgE is subsequently needed to mediate mast cell degranulation. IL-5 is pivotal for the growth, differentiation, recruitment, and survival of eosinophils, which act as the main effector cells responsible for chronic inflammation in allergic asthma [91, 92]. Respiratory epithelial cells, alveolar macrophages, and neutrophils are known to secrete CHI3L1 in human airways, and hence, this molecule has been attributed a role in airway inflammation [8]. A number of studies have shown that the levels of CHI3L1 are increased in asthma, where they correlate with disease severity and the degree of airway fibrosis [8, 30]. Ober et al. [93] studied a founder population of European descent and reported that Chi3l1 is not only a susceptibility gene for asthma, bronchial hyper-responsiveness, and reduced lung function but also elevated circulating CHI3L1 levels are a biomarker

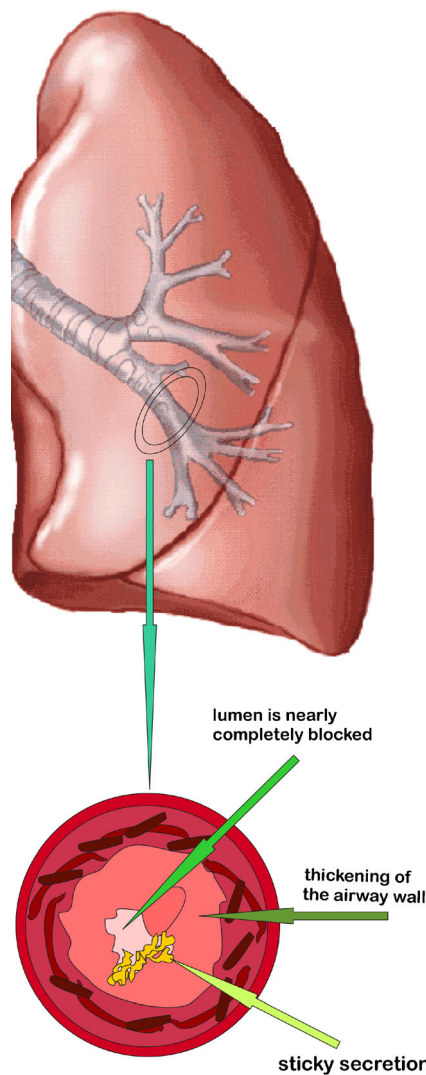


**Fig. 5** Representation of airways in asthma, demonstrating the thickening of the airway wall in asthma due to injury, chronic inflammation, and remodeling of epithelium and subepithelial tissue. In a case of fatal asthma, the lumina are completely blocked by sticky secretions composed of mucus admixed with inflammatory exudates [85]

for asthma and decline in lung function. Genetic variation in CHI3L1 showed to influence serum YKL-40 levels, and it was associated with several SNPs in CHI3L1 gene. They also found a linkage disequilibrium between studied SNPs. The role of CHI3L1 in the pathogenesis of asthma attracts the attention of many collaborative groups. Chupp et al., in an animal model of asthma, showed that chitinases were important effector molecules in the airway inflammation [8]. In this study, acidic mammalian chitinase, a true chitinase was shown to play a critical role in inflammation driven by Th2 cells and was expressed in an exaggerated manner in tissues from patients with asthma. By measuring circulating CHI3L1 levels in three cohorts, this group also established that CHI3L1 levels were increased in the lung and circulation of patients with severe asthma and correlate with the severity of disease.

Thereby, they reasoned that CHI3L1 could either be a cause or marker for asthma. Moreover, Kuepper et al. also showed that its concentrations predominantly increased at the site of allergen deposition in response to allergen challenge. They measured CHI3L1 concentrations in serum before and 24 h after segmental allergen challenge; moreover, they measured CHI3L1 levels in bronchoalveolar-lavage (BAL) fluid 10 min and 24 h after segmental allergen challenge. CHI3L1 levels in BAL revealed to be positively correlated with eosinophil counts 24 h after allergen challenge [94]. The expression level of CHI3L1 was increased during Th2 inflammation [74]. The activation of Th2 lymphocytes and their subsequent secretion of interleukin IL-13 are well-established key responses in the pathogenesis of allergic airway inflammation [9]. Induction of IL-13 secretion results in the alternative activation of alveolar macrophages, which are reported to play a role in the propagation of inflammation [10].

### Mechanism of Action

Most of the data regarding the role of CHI3L1 in asthma have been obtained by studying the biology of its homolog protein in null mice. BRP-39 (the murine orthologue of CHI3L1/YKL-40) has been associated with a number of diseases characterized by inflammatory and tissue remodeling responses [48]. Recently, many studies have focused on the regulatory function of BRP-39/YKL-40 in asthma and other allergic diseases. Lee et al. found that YKL-40 plays a novel regulatory role in the initiation and effector phases of Th2 inflammation and remodeling in mice [11]. In Th2 inflammation, BRP-39/YKL-40 are involved in multiple stages of allergic responses and regulate sensitization and Th2 cytokine effector functions by stimulating dendritic cell (DC) accumulation and activation [11]. The defects in antigen sensitization and Th2 inflammation in BRP-39 null mutant mice can be explained by a significant decrease in the numbers of myeloid and plasmacytoid dendritic cells and the ability of these cells to be activated after antigen exposure. The hypothetical regulatory pathways of BRP-39 in allergic inflammation and tissue remodeling response have been illustrated in Fig. 6 [95].

To determine if BRP-39 is regulated by Th2 inflammation, Elias et al. compared the expression of BRP-39 in the lungs from control mice and mice that had been sensitized and challenged with the chitin-free antigen OVA (ovalbumin). The study demonstrated that the levels of BRP-39 mRNA and protein were significantly increased after OVA sensitization and challenge. This was readily detected 24 h after aerosol OVA exposure and persisted at all time points that were assessed. This induction was not seen in mice that received a saline aerosol after OVA-plus-aluminum hydroxide (alum) sensitization or an OVA aerosol after saline sensitization. In all cases, BRP-39 was induced predominantly in airway epithelial cells and alveolar macrophages [11].

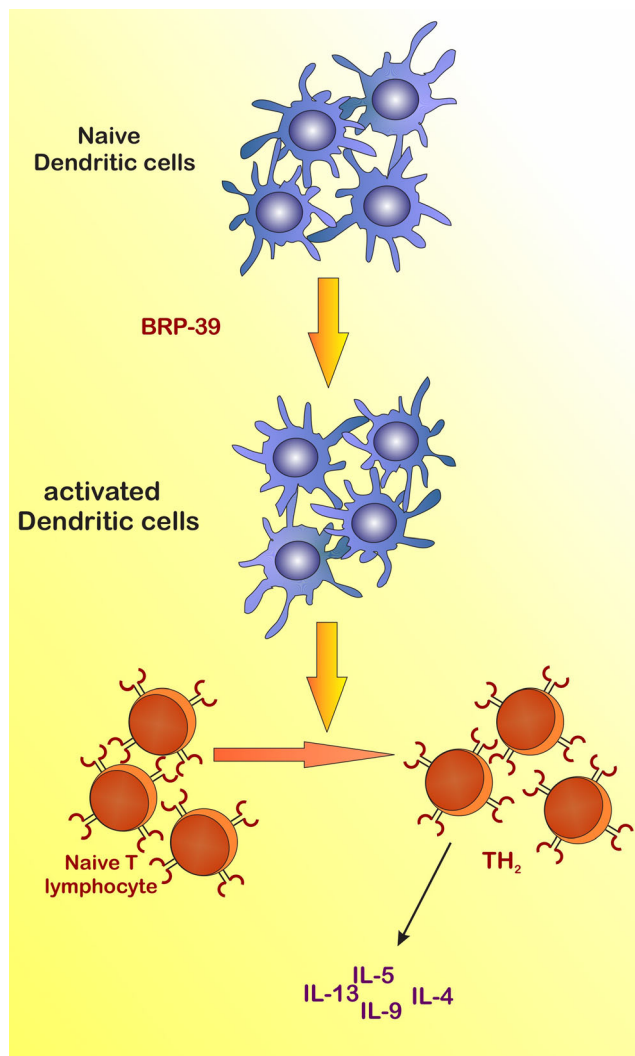


**Fig. 6** The proposed regulatory pathway of BRP-39 in allergic inflammation and tissue remodeling. Macrophages and epithelial cells are the primary cells expressing BRP-39 in the lung after allergen sensitization and challenge. BRP-39 increases the dendritic cell numbers in the lung and further activates and leads to enhanced Th2 polarization. BRP-39 also increases Th2 cells by reduction of T cell apoptosis or increase of cell survival. TGF- $\beta$  and other similar growth factors produced by Th2 cytokine stimulation leads to airway or alveolar remodeling. BRP-39 and Th2 cytokines such as IL-13 or IL-4 further contribute to the production of BRP-39 via regulation of cell death responses or alternative macrophage activation [95]

### Single Nucleotide Polymorphism CHI3L1 and Asthma

Given the appreciation of asthma as a heterogeneous and complicated disease of dysregulated immunity and its connection to atopy and allergic disease, many genes have been studied in order to obtain clues regarding its initiation and development [96•]. Studies such as genome-wide association study of serum CHI3L1 levels of Caucasian and Asian populations demonstrated that Chi3l1 is a risk factor gene with promoter polymorphisms that correlate with the levels of circulating CHI3L1, asthma prevalence, and abnormal lung

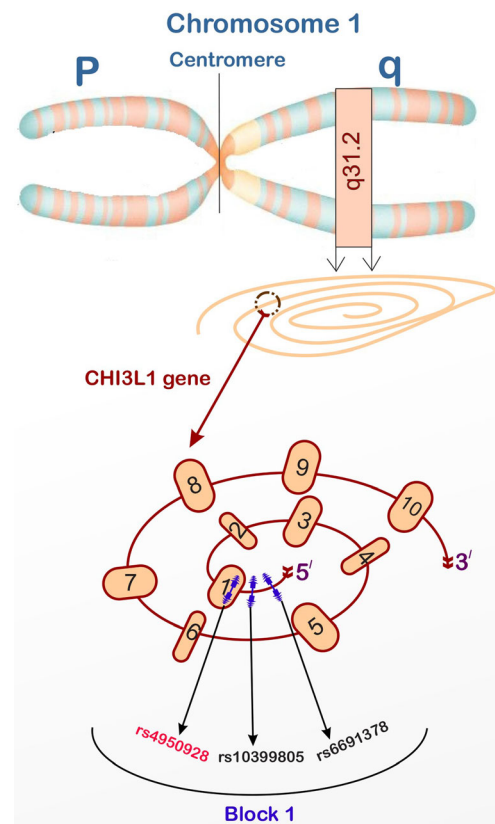


**Fig. 7** CHI3L1 coding gene which is located on q31.2 region on chromosome 1 contains 10 exons and several dominant SNPs relevant to asthma. Further investigations also showed linkage disequilibrium

function [93, 97]. Association of many polymorphisms of the CHI3L1 gene with asthma and atopic conditions has been studied widely in many populations.

Wang et al. successfully showed that CHI3L1 polymorphisms rs1538372 and rs10399931 can be used as genetic markers for predicting asthma risk in the Taiwanese population [12•], and -131 C  $\rightarrow$  G (rs4950928) polymorphism is one of the most studied SNPs of CHI3L1 gene with asthma and its severity. A genome-wide association study of asthma revealed that (SNP) rs4950928 in the promoter region of CHI3L1 is associated with serum levels of YKL-40 in western European and American populations [6, 93], and another study illustrated that the rs4950928 G allele is significantly associated with asthma susceptibility in a population-based study of 6514 Danish adults [98]. SNP rs4950928 causes a disruption of the binding site for transcription factors leading to a reduction in basal YKL-40 expression. Moreover, diminishing serum YKL-40 levels, the variant -131 G allele is also associated with protection against asthma, bronchial hyper-responsiveness, and reduced lung function [93]. A linkage disequilibrium (LD) plot of the examined variations of CHI3L1 shows that the two functional promoter SNPs of CHI3L1 rs4950928 and rs10399931 are in close LD

( $r^2 = 0.80$ ) [98]. However, there are other SNPs in the CHI3L1 coding gene shown to be related to asthma conditions (Fig. 7).

## Discussion and Conclusions

Association of CHI3L1 as a chitinase-like protein to allergic diseases such as asthma and a wide range of cancers has put it in the center of attention. In such conditions elevated levels of CHI3L1 are positively correlated with severity; a unique property making it a biomarker in the field of translational medicine. However, it is not a specific diagnostic marker to a certain disease.

Despite the recent progress in CHI3L1 research, still, there are lots of aspect of relationship between CHI3L1 and asthma to be clarified. First, what are the physiologically relevant CHI3L1 protein and carbohydrate-binding partners? The only identified protein-binding partner of CHI3L1 is type I collagen, and this interaction is unlikely to initiate intracellular signaling pathways. Second, how does CHI3L1 participate in tissue remodeling? Surprisingly, the mechanisms via which the CHI3L1 mediates its biologic effects are poorly understood. In addition to role of cytokines and other mediators in initiation and development of asthma, there are considerable challenges remaining in our understanding of its genetic underpinnings. The incredibly large quantity of data collected to date only explains a fraction of the heritability of asthma.

Lifestyle changes in industrialized countries have led to a decrease of the infectious burden, which are associated with the rise of allergic and autoimmune diseases, according to the “hygiene hypothesis.” Based on the hygiene hypothesis, parallel to decreasing the contact with such pathogens due to improvements in lifestyle, the prevalence of immune disorders such as allergic asthma increases. Many pathogens, for instance parasitic helminths, benefit from chitin in their cellular structure for various reasons such as structural rigidity. The high prevalence of asthma among people working with chitinous substances, such as crabs and fungi, is the strongest piece of evidence that chitin may be acting as an allergen (reviewed in Brinchmann et al. [99]).

Host immune responses to chitin and its degrading enzymes chitinases consist of two aspects: firstly, chitin itself is a molecule capable of orchestrating immune responses in mice resulting in the tissue accumulation of IL-4-expressing innate immune cells, including eosinophils and basophils [100]. Reese et al. also show the effect is ameliorated when the chitin was pre-treated with acidic mammalian chitinase, suggesting a protective role for chitinases. A similar role may also be envisioned for CHI3L1, despite lacking enzymatic activity.

In accordance with this finding, transgenic mice capable to over express AMCase in the lungs after chitin challenging with *Nippostrongylus brasiliensis* showed no histologic abnormalities in their lungs.

However, at the same time, both true chitinases and CLPs are capable to orchestrate and trigger allergic immune responses in organisms in the absence of chitin.

CLPs, in spite of lacking the ability to degrade chitin, are capable molecules to inhibit oxidant-induced lung injury, augment adaptive Th2 immunity, regulates apoptosis, stimulating alternative macrophage activation, and participating in pathophysiological events such as fibrosis and wound healing.

## Compliance with Ethical Standards

**Conflict of Interest** Drs. Elieh Ali Komi, Kazemi, and Bussink declare no conflicts of interest relevant to this manuscript.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of major importance

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