Published in final edited form as:
*Curr Opin Allergy Clin Immunol*. 2009 October ; 9(5): 401–408. doi:10.1097/ACI.0b013e3283306533.

# The Chitinase and Chitinase-Like Proteins: A Review of Genetic and Functional Studies in Asthma and Immune-Mediated Diseases

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## Abstract

**Purpose of Review—**This review provides an overview of the chitinase and chitinase-like proteins, chitotriosidase (CHIT1), YKL-40, and acid mammalian chitinase (AMCase), and to summarize the genetic studies of asthma and immune mediated diseases with polymorphisms in the genes encoding these proteins: *CHIT1*, *CHI3L1*, and *CHIA*, respectively.

**Recent Findings—**Polymorphisms in the *CHIT1*, *CHIA*, and *CHI3L1* genes influence chitotriosidase enzyme activity, AMCase activity, and YKL-40 levels, respectively. Regulatory SNPs in *CHI3L1* were also associated with asthma, atopy, and immune-mediated diseases, and nonsynonymous SNPs in *CHIA* were associated with asthma. No *CHIT1* polymorphisms, including a common nonfunctional 24-bp duplication allele, have been associated with asthma.

**Summary—**These genes represent novel asthma susceptibility genes. Variation in *CHI3L1* and *CHIA* have been associated with asthma risk. Polymorphisms in *CHIT1* have not yet been associated with asthma, but few studies have been reported. Given that chitotriosidase is the major chitinase in the airways and a common nonfunctional allele is present in many populations, additional studies of this gene are also warranted. Lastly, studies of all three genes need to be conducted in populations of diverse ancestries.

### Keywords

Chitotriosidase; *CHIT1*; YKL-40; *CHI3L1*; AMCase; *CHIA*

## Introduction

Chitin is an abundant, naturally occurring polysaccharide composed of N-acetylglucosamine repeats [1,2]. Following cellulose, chitin is the second most abundant polysaccharide in



Financial Disclosure: Dr. Chupp receives speaker fees from GlaxoSmithKline and Astra Zeneca, and grant support from Novartis and Gilead.

nature functioning as a major structural polymer in many lower life forms including the cell walls of bacteria and fungi, the shell of crustaceans such as crabs and shrimp, and the exoskeletons of arthropods such as dust mites and cockroaches [3–5]. In addition to this key structural function, chitin is also an important nutritional source for many organisms. When deposited on the ocean or forest floor chitin is quickly degraded by bacterial chitinases into glucosamine and then consumed [6,7]. Environmental levels of chitin have been estimated, but to date no studies have examined the levels in domestic samples such as house dust or how chitin levels vary between climates and geography [8]. Chitin can be both pro- and anti-inflammatory in animal models: orally and intranasally delivered chitin inhibits antigen driven Th2 inflammation whereas chitin enhances Th2 inflammation when delivered to the lung in isolation. These paradoxical results suggest that there is a complex interaction between environmental chitin exposure and the pathogenesis of allergic diseases [1].

Chitinases are a family of evolutionarily conserved hydrolases characterized by their ability to cleave chitin [9]. These enzymes have been studied extensively in lower life forms where they function to control chitin homeostasis in the organism and degrade chitin present in the surrounding environment. Recent studies have identified functional mammalian chitinase genes and several chitinase-like genes, which code for proteins that bind to chitin, but do not catalyze it [10,11]. Evidence in animals and humans suggests that both chitinases and chitinase-like proteins are potent up- or down-regulators of the innate immune response by interacting with and degrading chitin (depending on the size of chitin fragments present in the airway) and independent of chitin (and possibly chitinase activity) by modulating immune responses. In this context, they may be critically juxtaposed between the environment and the immune response by controlling the host's exposure to chitin and modulating the host's inflammatory response (Figure 1) [1,12,13].

## Chitinases, Chitinase-Like Proteins, and Asthma

The mammalian chitinases that have been most studied and implicated in the pathogenesis of asthma are chitotriosidase (CHIT1) and Acidic Mammalian Chitinase (AMCase), both true chitinases, and YKL-40 (also referred to as human cartilage gp-39 and chitinase 3-like 1 protein), a chitinase-like-protein that has the ability to bind chitin, but not to degrade it. CHIT1 and AMCase have similar ezymatic activities and are both capable of cleaving chitin polymers into oligosaccharides of varying sizes (endochitinase activity) and releasing glucosamine monosaccharides from the end of a chitin polymer (exochitinase activity) [14]. AMCase is called "acidic" because its chitinase activity is highest at a pH of ~2.0, whereas chitotriosidase is most active at a pH of 4.0–5.0 [15]. The ability of these enzymes to degrade chitin is routinely measured in a chitinase activity bioassay that utilizes fluorescently labeled chitin as a substrate [16].

The first link between asthma and the chitinase family of proteins was based on observations made on AMCase in animal models of asthma. In these studies, Zhu et al. examined transgenic mice that were engineered to overexpress IL-13 in the airway [17]. These mice expressed exaggerated levels of AMCase in the lung and spontaneously developed Th2 inflammation in the airway that was inhibited when the mice were treated with the non-specific chitinase inhibitor, allosamidin. Subsequently, in a different model of Th2 inflammation, Reese et al. more recently demonstrated that AMCase down regulated the inflammatory response caused by the delivery of chitin to lung [18]. These studies suggested that AMCase has complex effects and is pro-inflammatory downstream of IL-13, but can modulate chitin-induced Th2 inflammatory responses in the lung. Human studies on AMCase are limited, but a recent study of AMCase in epithelial cells cultured from patients with chronic sinusitis demonstrated that patients with refractory disease expressed lower levels of AMCase compared to nasal epithelial cells cultured from patients with responsive

disease [19,20], and a genetic study further implicated AMCase in asthma risk [21] (described below).

The other true chitinase, chitotriosidase is readily measured in serum and plasma in both healthy and disease states. Because chitinase activity in the periphery (at non-acidic pH) is entirely attributable to chitotriosidase, measurements of CHIT1 activity in serum or plasma is considered a surrogate for measuring chitotriosidase protein, although studies correlating activity and protein levels are limited. Chitinase activity is markedly elevated in the lysosomal storage disorder Gaucher's disease, where it is used as a marker of response to enzyme replacement therapy [22]. Chitotriosidase activity levels have also been shown to be elevated in thalassemias, arteriosclerosis, coronary artery disease, and to correlate with cardiovascular events in population studies [19,23–25]. It is also elevated in bronchoalveolar lavage (BALF) in both sarcoidosis and interstitial pulmonary fibrosis [26,27]. Recently, Seibold and colleagues demonstrated that in humans, chitotriosidase is the major chitinase expressed in the airway where it is elevated in smokers, but not mild asthmatics [28].

The chitinase-like-protein, YKL-40, was first discovered in mouse breast cancer cells and named BRP-39. YKL-40 binds chitin, but unlike CHIT1 and AMCase, nearly all studies of this chitinase-like protein focus on its endogenous "chitin independent" effector functions. In humans, YKL-40 appears to important in the homeostasis of many organs. It is produced by monocytes, macrophages, neutrophils, cultured chondrocytes and synovial cells, where it regulates cell proliferation and survival and has mitogenic effects on human skin, lung fibroblasts, and synoviocytes [29–33]. Similar to CHIT1, YKL-40 is present in the circulation of healthy subjects and is elevated in a number of human diseases. Increased circulating levels of YKL-40 have been observed in malignancies, such as lung cancer [34,35], breast cancer [36], and melanoma [37,38], in infections, such as severe purulent meningitis and community-acquired pneumonia [39,40], and in rheumatoid arthritis and osteoarthritis [41–43]. YKL-40 levels correlate with disease activity in rheumatoid and osteoarthritis and with tissue remodeling in cirrhosis and rheumatoid arthritis [33,44]. Animal studies also suggest that this protein is a key immune regulator. In these studies, the YKL-40/BRP-30 knockout mice were generated and found to have blunted Th2 inflammatory responses and increased inflammatory cell apoptosis when subjected to ovalbumin sensitization and challenge. This phenotype is rescued when YKL-40/BRP-39 is over-expressed in the airway of the knockout mice [45].

Based on the observation that the murine homologue of YKL-40 was expressed downstream of IL-13, we hypothesized that YKL-40 levels in the peripheral circulation would be increased in individuals with asthma and would increase with disease severity [46,47]. To examine this hypothesis we performed a cross-sectional analysis of samples from an established cohort of asthmatic subjects and controls from the Yale Center for Asthma and Airways Disease, a second sample of asthmatic subjects and controls from the University of Wisconsin, and a third sample of asthmatic subjects and controls from the University of Paris. These studies showed that YKL-40 levels were significantly higher in the serum from asthmatic compared to non-asthmatic subjects, and correlated with asthma severity. In addition, serum levels of YKL-40 were positively correlated with expression of YKL-40 in airway biopsies (by immunohistochemistry) and negatively correlated with sub-basement membrane thickening and FEV1. These observations suggested that YKL-40 plays a role in asthma pathogenesis, which has been supported by the animal studies described above and genetic studies (described below).

## Genetic Studies of Chitinase and Chitinase-Like Genes

Although the number of recognized chitinase and chitinase-like genes is expanding, genetic studies have thus far been limited to only three members of this family, including the genes encoding chitotriosidase (*CHIT1*) and chitinase 3-like 1 (*CHI3L1*; also referred to as YKL-40 and cartilage glycoprotein-39) on chromosome 1q32.1, and acid mammalian chitinase (AMCase) (*CHIA*) on chromosome 1p13.1-p21.3. These studies are reviewed below.

### *CHIT1* Polymorphisms, CHIT1 Activity, and Immune-Related Phentoypes

The *CHIT1* gene encompasses 12 exons, spanning ~20 kb and encoding multiple splice forms. Nearly all of the genetic studies to date have focused on variation in the coding region, and primarily on a 24-bp duplication polymorphism in exon 10. The 24-bp duplication allele (rs3831317) results in a nonfunctional protein and is consistently associated with lack of CHIT1 enzymatic activity [25,48–50]. Curiously, this allele shows a clinal distribution worldwide with the highest frequencies in East Asian populations (0.45–0.64) [49], followed by Middle Eastern and Indian populations (0.35–0.37) [49], European populations (0.10–0.25) [25,49–51], Morocco (0.20) [51], and sub-Saharan Africa (0–0.07) [49,50]. The clinal distribution and divergent frequencies of the duplication allele in worldwide populations led several investigators to propose that the frequencies of the duplication (null) allele reflect the action of natural selection acting on this locus [49–51]. However, the population genetic and disease association studies to date do not provide strong or consistent support for this hypothesis. Therefore, the intriguing frequency distribution of this nonfunctional allele remains largely unexplained.

CHIT1 activity and/or *CHIT1* polymorphisms have been examined for associations with cardiovascular disease [25,48,51], asthma and atopy [49,52], and infectious conditions, including malaria [50,51], Gram-negative bacterial infections [53], and tuberculosis (TB) [49]. The results of association studies of the *CHIT1* polymorphisms are summarized in Table 1. In general, the 24-bp duplication allele was not associated with the diseases studied, with the following possible exceptions. Lee and colleagues reported an association between the 24-bp duplication and positive TB tests in Europeans (188 Ashkenazi Jewish subjects with a positive test and 492 controls subjects; allele $p = 0.0078$, genotype $p = 0.010$) but not in Asians (368 subjects with a positive test and 563 control subjects) and an association with three or more atopic diseases (asthma, allergic rhinitis, atopic dermatitis, allergies to drugs, cosmetics or food, and frequent sneezing, watering, or nasal congestion) in Asians (genotype $p = 0.028$) but not in Europeans [49]. Europeans who were homozygous for the 24-bp duplication were more likely to have a positive TB test and Asians who were homozygous for the 24-bp duplication were less likely to have three or more atopic diseases. The poor phenotype definition and modest p-value for the association with “atopy”, the inability to replicate either association in a second ethnic group, and the lack of correction for multiple testing, raise the possibility that the reported associations are false positive results. Furthermore, there were no descriptions of the matching of cases and controls for local ethnicity and, given the marked differences in the 24-bp duplication allele frequency between populations, it is possible that the observed associations resulted from imbalances in the ethnic composition of their case and control subjects [54,55].

Lehrnbecher et al. examined polymorphisms in eight genes in innate immune pathways, including *CHIT1*, in children with acute myeloid leukemia (AML) to determine whether variants in these genes contribute to infectious complications during therapy for acute AML [53]. They reported an association between the *CHIT1* 24-bp duplication allele and risk for Gram-negative bacterial infection ($p = 0.033$) in 168 North European Caucasian children (24 children with infection, 144 children without infection). The risk of bacteremia with Gram-

negative organisms was higher in children who were homozygous for the duplication (OR 2.78, 95% CI 1.05–7.4). As with the Lee study discussed above, there was no correction for multiple testing and no discussion of the specific ethnic composition of each group.

Single nucleotide polymorphisms (SNPs) in the *CHIT1* gene were investigated in two studies [49,52]. Bierbaum et al. studied the 24-bp duplication and two nonsynonymous SNPs, Gly102Ser in exon 4 (rs2297950) and Ala442Gly/Val in exon 12 (rs1065761), in 322 German children with asthma and 270 (non-phenotyped) German controls [52]. Neither the individual polymorphisms (Val442 was not present in the population) nor haplotypes comprised of the three polymorphisms were associated with asthma. The authors concluded that this gene does not play a major role in asthma susceptibility, although no one has thoroughly surveyed variation in this gene in populations characterized for asthma or related phenotypes.

Lee et al. genotyped the same three polymorphisms and an additional nonsynonymous SNP in exon 10, Gly354Arg (rs9943208), in individuals representing worldwide samples with CHIT1 activity measurements [49]. The mean activity levels of homozygotes for the non-duplicated (normal) allele was higher in Europeans (mean level = 2.69 mU/ml, N=202) compared to Asians (mean level = 0.86 mU/ml, N=114; $p < 0.0001$) or to Africans (mean level = 2.57 mU/ml, N=230; $p < 0.0001$), leading the authors to hypothesize that other variation in this gene influences CHIT1 activity levels. To address this hypothesis they sequenced the *CHIT1* coding region and proximal promoter in 11 subjects who were homozygous or heterozygous for the normal (non-duplicated) allele but had little or no CHIT1 activity. They identified the three coding SNPs described above and one promoter polymorphism (−1432A/G) that they did not study further. The frequencies of the Ser allele at the Gly102Ser SNP were similar in European (0.27), African (0.26), and Asian (0.24) populations and this SNP was not associated with CHIT1 activity in any of the populations. The Arg allele at the Gly354Arg SNP (rs9943208) was absent in Asians and very low frequency in Europeans (0.002), but present at a frequency of 0.09 in Africans, in whom this SNP was associated with decreased CHIT1 activity ($p<0.0001$). The Gly allele at the Ala442Gly/Val occurred at frequencies of 0.11 in Europeans and Africans but was less frequent in Asians (0.04), while the Val allele occurred at frequencies of 0.08 in Africans, 0.003 in Europeans, and was absent in Asians. At this SNP, the Val allele was associated with lower CHIT1 activity compared to the Ala and Gly alleles in Africans although the differences were only significant in comparisons to the Ala allele ($p = 0.0013$), like due to the small number of individuals with Gly/Gly, Gly/Val, and Val/Val genotypes. All analyses were performed only in individuals who were homozygous for the non-duplication (normal) allele, therefore suggesting that additional coding variation in the *CHIT1* gene influences CHIT1 activity in Africans.

In summary, the *CHIT1* gene harbors variation that significantly impacts chitotriosidase enzymatic activity. However, none of these variations, including the 24-bp duplication (null) allele, has been shown to have a significant effect on susceptibility to asthma or atopic diseases [49,52], lipids or coronary artery disease [48,51], or resistance to malaria [50]. On the other hand, associations between the 24-bp duplication with Gram-negative bacterial infection in children with AML [53], with TB positive tests [49], and possibly with other infectious diseases, should be considered further.

### *CHI3L1* Polymorphisms, YKL-40 levels, and Inflammatory Diseases

The *CHI3L1* gene encompasses 10 exons (Figure 2A), spanning ~8 kb and encoding multiple splice forms. Six studies have investigated associations between one or more polymorphisms in this gene and YKL-40 levels and disease phenotypes (Figure 2B). Three SNPs in the promoter region of *CHI3L1*, −131C/G (rs4950928) [56–58], −247C/T

(rs10399805) [59], and −329G/A (rs10399931) [60] have been associated with serum levels of YKL-40. Other variants in the gene, other than those in linkage disequilbrium (LD) with these variants [58], have not been associated with any phenotypes investigated, including serum YKL-40 levels (Figure 2).

Two of the promoter SNPs, −131C/G and −247C/T, were shown to be functional, with −131G and −247C each associated with decreased transcription in luciferase assays and lower levels of serum YKL-40 [56,59]. These two SNPs are not in LD in European or Asian populations, although they show modest LD ($r^2$=0.46) in Africans (Figure 2C). The −131G allele (on the forward strand) has been associated with risk for schizophrenia in Chinese [56], protection from asthma, bronchial hyperresponsiveness, and decreased lung function (but no effect on risk for atopy) in European and European Americans [58], and increased severity of hepatitis C-induced liver fibrosis in Europeans [57]. The association with asthma was not replicated in Korean children [59] or in European American children (using a SNP in strong LD with −131C/G) [61]. The −247T allele was associated with higher IgE levels and atopy (but not with asthma) in Korean children [59]. The association of this SNP with atopy was not replicated, although the association with YKL-40 levels was modest, in a study of European and European Americans [58]. On the other hand, this SNP was not associated with YKL-40 levels in Dutch [60] or Chinese [56] subjects after taking into account the associations with other SNPs.

The third promoter SNP, −329G/A, is in LD with the −131C/G SNP in Europeans ($r^2$=0.84) and Africans ($r^2$=0.69) but less so in Asians ($r^2$=0.24) (Figure 2C). Kruit et al. reported that the −329A allele was associated with lower serum YKL-40 levels, but not with sarcoidosis, in Dutch subjects [60]. Because of the strong LD between this SNP and the functional −131C/G SNP, and the lack of functional data on this SNP, it is possible that the association with YKL-40 levels reported in this study was due to the −131C/G SNP. The association between −329G/A and YKL-40 levels was not replicated by Sohn et al. in a study of Korean children [59].

In summary, at least two functional promoter polymorphisms in this gene influence serum YKL-40 levels and risk for common diseases in European and Asian populations. The associations of different functional SNPs with asthma in Europeans (−131C/G) [58] and atopy in Koreans (−247C/T) [59] is intriguing and suggests that the regulatory elements corresponding to each SNP may have tissue-specificity or the effects of these elements may be influenced by ethnic (genetic) background. Regardless of the reasons for these seemingly discrepant results, variation in this gene requires further study in well-phenotyped diverse population samples.

### *CHIA* Polymorphisms and Asthma

The *CHIA* gene encompasses 12 exons, spanning ~29 kb and encoding multiple splice forms. Bierbaum and colleagues first hypothesized that variation in *CHIA* may contribute to asthma susceptibility [21]. They sequenced the exons and promoter region of this gene in 30 German individuals and identified six SNPs in the promoter region and three SNPs in the coding region, two of which were nonysonymous. They genotyped the three coding SNPs, one promoter SNP, and three additional nonsynonymous SNPs from public databases, in 322 German children with asthma, 270 (non-phenotyped) German controls, and 565 German children without asthma (phenotyped controls). A nonsynonymous SNP, Lys17Arg (rs3818822), was significantly associated with asthma (p = 0.0031 vs. non-phenotyped controls; p = 0.0172 vs. phenotyped controls), as was a synonymous SNP in exon 4 (rs3818822) (p = 0.0197 vs. non-phenotyped controls; p = 0.0003 vs. phenotyped controls). These two SNPs were in strong LD with each other and may, therefore, represent one association signal [21]. The frequencies of 7-SNP haplotypes that occurred in their sample at

a frequency of ≥0.01 differed significantly between cases and controls ($p = 0.000039$ vs. non-phenotyped controls; $p < 10^{-10}$ vs. phenotyped controls; based on 100,000 permutations implemented in the program FAMHAP [62]). Interestingly, the promoter SNP (rs12033184) was not associated with asthma by itself but differentiated otherwise identical haplotypes that had divergent frequencies between the cases and the phenotyped controls. Overall, this study identified a nonsynonymous SNP, Lys17Arg, and possibly a promoter SNP (rs12033184), that are associated with risk for pediatric asthma in Germans and further implicates AMCase in asthma susceptibility.

Recently, Seibold et al. resequenced all exons in the *CHIA* gene in 72 African American, Puerto Rican, and Mexican subjects with asthma and identified 21 variants, including nine nonsynomymous SNPs [63]. Eight nonsynomymous SNPs were common in all three groups; the Lys17Arg SNP was not present in this study. Three nonsynonymous SNPs, Arg61Met, Asn45Asp, and Asp47Asn, clustered in the glycosyl hydrolase domain near the active site residues. These three SNPs and five other coding SNPs were genotyped in 176 African Americans, 602 Mexicans, and 798 Puerto Ricans. Haplotypes comprised of these SNPs that were protective against asthma were also associated with increased levels of chitinase activity *in vitro*. The investigators suggested that AMCase may function to limit the effects of chitin induced inflammatory responses.

## Conclusions

The chitinase and chitinase-like proteins represent a newly identify pathway of modulators contributing to inflammatory diseases. Both functional and genetic studies implicate YKL-40 and AMCase in the pathogenesis of asthma. At least two functional promoter variants in the *CHI3L1* gene, encoding YKL-40, have been associated with YKL-40 levels in addition to asthma, atopy, or other immune-related phenotypes (Figure 2). Thus, to date, this gene and protein have the most supporting evidence for contributing to inter-individual variation in disease risk. Two studies of the *CHIA* gene, encoding AMCase, have been reported, both showing associations between nonsynonymous SNPs and asthma, and one of those studies in addition showing functional effects of the associated variants on enzymatic activity of AMCase. Lastly, variation in the *CHIT1* gene has significant impact on enzymatic activity of chitotriosidase but, to date, genetic variation in this gene has not been associated with asthma or allergic phenotypes (Table 1). However, few studies have directly assessed associations between *CHIT1* polymorphisms and these phenotypes. The recent demonstration of CHIT1 as the major chitinase in the lung leads us to suggest that further studies of the 24-bp duplication and variation throughout this gene in populations well-characterized for asthma and allergy phenotypes are also warranted.

## Acknowledgments

The authors acknowledge NIH grants HL072414, HL070831, and HL085197 (C.O.) and HL095390-01 and UL1RR024146 (G.L.C.).

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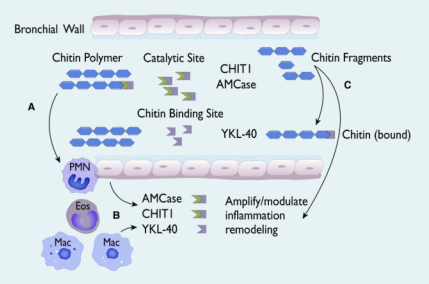


**Figure 1. The current paradigm of chitin and chitinases in the airway**

Chitin polymers from the decay of dust mites and other organisms are inhaled and deposited in the airway (A), where it initiates an innate immune response in the bronchial wall. Here they stimulate the production of cytokines, chitinases (AMCase, CHIT1), and chitinase-like-proteins (YKL-40) from neutrophils (NEU), Macrophages (MAC), and eosinophis (EOS) (B). These cytokines in turn drive inflammation and remodeling in the airway wall. Locally produced chitinases also degrade chitin polymers into smaller fragments or are bound by chitinase-like-proteins, such as YKL-40, which alter the biologic effects of the chitin fragments on the immune response (C).

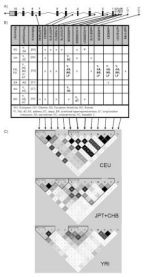


**Figure 2. *CHI3L1* gene structure, polymorphisms and LD patterns**

A) The *CHI3L1* gene is shown (not to scale). Coding exons are shown as black rectangles; untranslated exons are shown as gray rectangles. The relative location of SNPs in the 5' upstream region are shown; three promoter SNPs that have been associated with phenotypes and/or YKL-40 levels are shown in bold font. B) The six genetic studies of this gene are summarized. Small "x" indicates that the SNP was genotyped in the sample but not associated with YKL-40 levels or the phenotypes interrogated. Phenotype symbols in the box under each SNP indicates that associations were reported. C) LD pattern ($r^2$ values shown in each square) between 13 interrogated SNPs in European (CEU), Asian (JPT +CHB), and African (YRI) HapMap samples.

**Table 1**

Association studies between CHIT1 polymorphisms and CHIT1 enzyme activity and phenotypes

| Phenotype | Population | Polymorphisms[a] | Assoc. with CHIT1 activity | Assoc. with phenotype |
|---|---|---|---|---|
| Lipids [48] | European | 1 | Yes | No |
| CHIT1 activity [50] | Mediterranean and African | 1 | Yes | |
| Atherosclerosis [25] | Spanish | 1 | Yes | No |
| Gram-negative bacteria infection in AML [53] | N. European | 1 | Not assayed | Yes |
| Asthma [21] | German | 1,2,4 | Not assayed | No |
| Positive TB test [49] | Worldwide | 1–4 | Yes (1, 2, 3) | Yes (1, Europeans only) |
| Atopic diseases [49] | Worldwide | 1–4 | Yes (same as above) | Yes (1, Asians only) |
| Malaria [51] | Mediterranean | 1 | Not assayed | No |
| Coronary heart disease [51] | Corsica (French) | 1 | Not assayed | No |

AML, acute myeloid leukemia; Assoc., association; CHIT1, chitotriosidase.

[a]Polymorphisms: 1, 24-bp dup. (rs3831317); 2, A442G/V (rs1065761); 3, G354R (rs9943208); 4, G102S (rs2297950).