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CHIT1 Mutations: Genetic Risk Factor for Severe Asthma With Fungal Sensitization?

abstract



Fungi can exacerbate symptoms in patients with asthma. To our knowledge, genetic risk factors for fungal-associated asthma have not been described. We present here the cases of 6 children who carried the diagnosis of severe asthma with fungal sensitization, 3 of whom were treated with and responded clinically to itraconazole therapy. All 6 patients were heterozygous for a 24-base pair duplication in the *CHIT1* gene, which has been associated with decreased levels of circulating chitotriosidase and susceptibility to fungal infection. *Pediatrics* 2010; 126:e982–e985

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KEY WORDS

innate immunity, allergy, chitin

ABBREVIATIONS

ABPA—allergic bronchopulmonary aspergillosis

SAFS—severe asthma with fungal sensitization

IgE—immunoglobulin E

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Fungi have been linked to severe asthma in numerous studies.^{1–3} Anti-fungal treatment has been reported to improve clinical symptoms for some asthmatic patients with allergic bronchopulmonary aspergillosis (ABPA), allergic fungal sinusitis, and dermatophytic infections.^{4–7} Recently, Denning et al⁸ studied a population of patients with severe asthma with fungal sensitization (SAFS) whose symptoms improved during prolonged itraconazole therapy. Together, these observations suggest that ongoing fungal infection, recognized or not, can directly contribute to asthma symptoms.

The prevalence of persistent asthma is disproportionately high in certain urban areas including the Bronx, New York, partly because of sensitization against numerous environmental allergens.⁹ However, the precise role of fungi in this context is not known. Although environmental exposure to fungi is ubiquitous, only select patients seem to be affected significantly. Currently, there are few data regarding the susceptibility of certain asthmatic patients to fungi.

The *CHIT1* gene encodes mammalian chitotriosidase, a 50-kDa enzyme that catalyzes the cleavage of chitin polymers.¹⁰ Chitin is present in fungal cell walls, the exoskeletons of crustaceans and insects, and in parasites. Plants use chitotriosidase as an important innate defense mechanism against fungi, and mice that lack the enzyme demonstrate increased susceptibility to invasive fungal disease.¹¹ However, the precise role of chitotriosidase in humans has not yet been defined.

We describe here 6 Bronx children with apparent SAFS whose *CHIT1* genotype was determined. It is interesting to note that all 6 children carried a 24-base pair duplication in the gene that is associated with decreased levels and enzymatic activity of chitotriosidase.¹⁰

TABLE 1 Patients With SAFS

Patient No.	Age, y	Gender	FEV ₁ , % ^a	Peak IgE Level, IU/mL	Fungal Sensitization	<i>CHIT1</i> Genotype	Itraconazole Treatment
1	10	Male	72	24 760	Multiple ^b	Heterozygous	Yes
2	10	Male	75	8907	Multiple ^b	Heterozygous	Yes
3	9	Female	57	3777	<i>Aspergillus</i> , class 1	Heterozygous	No
4	7	Female	61	1206	<i>Aspergillus</i> , positive skin test	Heterozygous	Yes
5	10	Male	74	1972	<i>Penicillium</i> , class 2	Heterozygous	No
6	6	Male	NA	1869	<i>Aspergillus</i> , class 1	Heterozygous	No

At minimum, maintenance therapy with high-dose inhaled corticosteroids, long-acting β agonists, and leukotriene receptor antagonists failed to significantly improve the patients' condition. With the exception of patient 2, as described in the main text, they were not considered candidates for omalizumab therapy on the basis of age and IgE level. FEV₁ indicates forced expiratory volume in 1 second; NA, not applicable.

^a Average percent predicted over a 1- to 2-year period.

^b See main text for sensitization patterns.

CASE REPORTS

Patient 1

A 10-year-old boy with severe persistent asthma was maintained on high-dose inhaled corticosteroids, long-acting β agonists, and leukotriene receptor antagonists. Because of repeated hospitalizations, daily low-dose prednisone was initiated; however, his symptoms continued, and the results of his pulmonary-function testing continued to indicate obstruction (Table 1). His serum immunoglobulin E (IgE) level peaked at 24 760 IU/mL. Extensive evaluation revealed no alternate disease. Radioallergosorbent test results were positive for *Alternaria* (10.6 kU/L; class 3), *Cladosporium* (1.35 kU/L; class 2), *Penicillium* (0.8 kU/L; class 2), and *Aspergillus* (5.39 kU/L; class 3). Because all previous therapy had failed, the patient was started on itraconazole 100 mg by mouth twice daily. Within the first month of treatment, his IgE level decreased to 1862 IU/mL, pulmonary-function test results normalized, and systemic steroids were weaned, and the patient reported resolution of his symptoms. During treatment, he experienced only 1 exacerbation after acquiring an upper respiratory infection and was treated as an outpatient.

Genotype analysis, performed under an institutional review board–

approved protocol (including parental consent and patient assent) and using previously described methods,¹² revealed the 24-base pair duplication in *CHIT1* (note that the case of patient 1 was reported in greater detail,¹³ but his genotype was unknown and, thus, was not included in the original report).

Patient 2

A 10-year-old boy from Boston, Massachusetts, was diagnosed with severe persistent asthma. Despite treatment with high-dose inhaled corticosteroids, long-acting β agonists, and leukotriene receptor antagonists, he required frequent hospitalizations, monthly steroid bursts, and, eventually, chronic systemic steroids. Although his serum IgE level fell outside the dosing range for omalizumab ($\sim 10\,000$ IU/mL), the medication was initiated. He continued to exhibit daily symptoms and require frequent hospitalizations, and the omalizumab was discontinued. Similar to patient 1, extensive evaluation did not identify other diseases.

The patient relocated to the Bronx, where his symptoms continued and results of his pulmonary-function testing continued to indicate obstruction (Table 1). His peak IgE level at our institution was 8907 IU/mL, and results of a

radioallergosorbent test were positive for *Alternaria* (3.19 kU/L; class 2), *Cladosporium* (1.10 kU/L; class 2), *Penicillium* (1.39 kU/L; class 2), and *Aspergillus* (1.33 kU/L; class 2). After initiation of itraconazole 100 mg by mouth twice daily, his symptoms improved dramatically (decreased β -agonist requirement and successful weaning from systemic steroids). In comparison to patient 1, this patient's IgE level remained elevated (6000–8000 IU/mL) throughout the 6-month treatment. As with patient 1, genotype analysis demonstrated the patient to be heterozygous for the 24-base pair duplication in *CHIT1*.

Patients 3 Through 6

Because of our experience with patients 1 and 2, we sought to retrospectively identify similar children for determination of *CHIT1* genotype. We previously banked bronchoalveolar lavage fluid from children who underwent clinically indicated flexible bronchoscopy under an institutional review board–approved protocol (including consent for future studies). Among 16 surplus samples, we identified 4 from patients with apparent SAFS who, specifically, (1) were diagnosed with asthma and whose condition failed to respond to step 4 or greater therapy, (2) had an elevated serum IgE level, and (3) had fungal sensitization according to the results of radioallergosorbent or skin testing. All 4 were heterozygous for the *CHIT1* duplication.

Among this group, patient 4 fulfilled several diagnostic criteria for ABPA (elevated serum IgE level, peripheral eosinophilia, immediate reactivity to *Aspergillus* skin testing, and recurring infiltrates on chest radiographs). Because of her chronic dependence on systemic steroids (>6 months) and suspected diagnosis of ABPA, itraconazole was initiated. During treatment, the patient was successfully weaned

from her systemic steroid therapy. Because patients 3, 5, and 6 were managed before the Denning et al⁸ study and were not suspected to have ABPA, we did not have precedent for treatment. A summary of all the patients is provided in Table 1.

Follow-up

For patients who received itraconazole, we analyzed liver function monthly, and no abnormalities were found. Patients 1 and 2 experienced an exacerbation that required hospitalization soon after discontinuation of itraconazole. However, both patients continued to demonstrate sustained improvements in symptoms ~12 months after treatment while continuing their inhaled corticosteroids, long-acting β agonists, and leukotriene receptor antagonists. It is important to note that both families made environmental modifications to limit potential exposures (more stringent cleaning of moldy areas and relocation to a different household, respectively). Patient 4 relocated to Florida and was lost to follow-up. The nontreated patients were lost to follow-up before publication of the Denning et al⁸ study.

DISCUSSION

SAFS is a newly described subphenotype of severe asthma characterized by failure of step 4 or greater therapy, elevated serum IgE level, and fungal sensitization. Denning et al⁸ initially described this clinical entity and demonstrated itraconazole to be an effective adjunct treatment. We recently described a similar case of a child whose condition responded dramatically to itraconazole.¹³ Together, these reports suggest that SAFS may represent an underappreciated population of patients who could benefit from antifungal therapy. There currently are no known risk factors for SAFS. We have described here 6 children with appar-

ent SAFS, all of whom carried a mutation in *CHIT1*.

It is important to note that we modified the original definition of SAFS for our patients. In their initial article, Denning et al⁸ excluded *Aspergillus* as a potential contributor to disease because of overlap with ABPA. A well-described complication of asthma, ABPA is rarely diagnosed in children (as was the case for our patients), who seldom fulfill the recommended diagnostic criteria including positive skin-testing results, serum precipitants against *Aspergillus*, elevated IgE level (>1000 IU/mL), *Aspergillus*-specific IgE, peripheral eosinophilia (>10%), recurring infiltrates on chest radiograph, and central bronchiectasis.⁴ Their rationale for excluding these patients was to avoid overinterpretation of results, because patients with ABPA have been shown to respond to itraconazole. Indeed, inclusion of these patients may have strengthened the original data. For similar reasons, they excluded patients with serum IgE levels of >1000 IU/mL. As such, none of our patients would have been candidates for inclusion, because all of them demonstrated serum IgE levels of >1000 IU/mL, and most of them demonstrated sensitivity to *Aspergillus*. Nevertheless, we chose to apply the SAFS diagnosis to these patients because they did not fulfill diagnostic criteria for ABPA but could potentially benefit from itraconazole therapy. Given the positive response we observed, it seems reasonable that application of our modified criteria (ie, inclusion of patients with IgE levels of >1000 IU/mL and sensitivity to *Aspergillus*) could be useful for other patients. In reviewing 82 serum IgE levels in Dr Vicencio's previous asthma clinic, we found the average value to be 725 IU/mL, and 15% of these levels were >1000 IU/mL; of 42 fungal radioallergosorbent tests analyzed, 25% demonstrated sensitiv-

ity to *Aspergillus* (unpublished observations). It is interesting to speculate whether such patients might benefit from itraconazole using our modified SAFS guidelines. It is important to consider the possibility that such patients are simply in the early stages of ABPA and might eventually fulfill diagnostic criteria.

All 6 patients in our series (95% confidence interval: 0.52–1.00) were heterozygous for a 24-base pair duplication in the *CHIT1* gene. In previous studies, the heterozygous and homozygous frequencies of the *CHIT1* duplication were 35% and 6%, respectively.¹² However, the relative proportion of these genotypes in our population is unknown. A recent review of chitinases and asthma highlighted the lack of an association with *CHIT1*, but the investigations on which this review was based did not account for fungal sensitization.¹⁰ Indeed, the authors acknowledged the potential role of *CHIT1* mutations and susceptibility to infections. It is important to note that chitinolytic activity is significantly dimin-

ished in both the heterozygous and homozygous states.¹⁰ Because plants and animals use chitotriosidase as a defense mechanism against fungi, it seems reasonable that the enzyme serves a similar purpose in humans and, hence, that deficiency contributes to susceptibility. However, because the human immune system possesses redundant mechanisms, *CHIT1* mutations may become relevant only when individuals are exposed to high levels of fungi or, perhaps, in states of immune compromise. Last, chitotriosidase is the predominately active chitinase in the human lung, which is typically the site of entry for many environmental fungi.¹⁴ Thus, although *CHIT1* may not specifically contribute to the development of asthma, mutations may increase the risk of pulmonary fungal infection or colonization, thereby facilitating sensitization and complicating lung disease.

CONCLUSIONS

We have identified 6 children who fit a modified diagnosis of SAFS and are heterozygous for a 24-base pair dupli-

cation in the *CHIT1* gene. We hypothesize that the *CHIT1* duplication may be a risk factor for SAFS and similar diseases including ABPA and allergic fungal sinusitis but acknowledge that large controlled studies are indicated. Immediate studies could focus on the prevalence of SAFS in different geographic locations, association with *CHIT1* or other genes, and optimal treatment strategies.

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