

# House Dust Mite Subcutaneous Immunotherapy Does Not Induce New Sensitization to Tropomyosin: Does It Do the Opposite?

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## ■ Abstract

*Background:* It is still uncertain whether house dust mite (HDM) tropomyosin present in allergen extracts can cross-sensitize patients receiving subcutaneous immunotherapy (SCIT) and thus induce food allergy.

*Objectives:* Our aim was to assess whether new sensitization to tropomyosin occurred during HDM-SCIT, and, if so, whether it was clinically relevant.

*Patients and Methods:* The study sample comprised 56 HDM-allergic patients treated with SCIT using HDM extract. All patients were screened for specific IgE (sIgE) to mite tropomyosin (rDer p 10) before and after SCIT. In patients with a positive result, we also monitored the dynamics of sIgE to rDer p 10 and shrimp tropomyosin (rPen a 1) at several time points. The levels of sIgE were measured using the CAP System fluorescent-enzyme immunoassay.

*Results:* sIgE to tropomyosin was found in only 5 patients, 3 of whom expressed low and clinically irrelevant levels of sIgE to Der p 10, while sIgE to Pen a 1 was not found. The remaining 2 patients expressed sIgE to both tropomyosins. In the first, the initial increase and subsequent decrease resembled the dynamics of the IgE antibodies usually seen in SCIT patients and were never accompanied by seafood-induced symptoms. In the other, a decrease in levels of sIgE to both tropomyosins resulted in the complete loss of his reactivity toward seafood.

*Conclusions:* Immunotherapy using HDM extracts does not induce clinically relevant sensitization to tropomyosin. In certain cases of combined mite and seafood allergy, treatment may even lead to the improvement of food allergy symptoms. The levels of sIgE to Der p 10 and Pen a 1 may be useful monitoring markers.

**Key words:** House dust mite. Seafood. Sensitization. Specific immunotherapy. Tropomyosin.

## ■ Resumen

*Antecedentes:* Es un hecho incierto que la tropomiosina presente en los extractos alergénicos puede sensibilizar a los pacientes que reciben inmunoterapia Ag-específica e inducir alergia alimentaria.

*Objetivo:* El objetivo de este estudio fue evaluar si una inmunoterapia subcutánea con extractos de ácaros del polvo de casa puede inducir a una sensibilización a tropomiosina y si esta podría ser clínicamente relevante.

*Métodos:* Se incluyeron en el estudio 56 pacientes alérgicos al ácaro del polvo de casa, tratados con un extracto de ácaros. En todos los pacientes se analizó la IgE esp frente a tropomiosina del ácaro (rDer p 10) antes y después de la IT. En los pacientes con resultado positivo también se monitorizó la IgE esp frente a las tropomiosinas del ácaro y de la gamba (rPen a 1) en varios tiempos, mediante CAP-System FEIA.

*Resultados:* En cuanto a los resultados obtenidos, la IgE esp frente a tropomiosina fue positiva únicamente en 5 pacientes, tres de los cuales mostraban valores bajos y clínicamente irrelevantes de IgE esp frente a Der p 10 y no se encontró en ningún caso IgE esp positiva frente a Pen a 1. Los otros dos pacientes mostraron IgE esp positiva a ambas tropomiosinas. En el primero de ellos se observó un incremento inicial y una posterior disminución tras la IT, dinámica similar a la observada habitualmente con los anticuerpos IgE en los pacientes sometidos a inmunoterapia subcutánea y que nunca se acompañaba de síntomas con la ingesta de marisco. En el otro caso, la disminución de la IgE esp frente a ambas tropomiosinas resultó en la completa pérdida de reactividad frente a marisco.

*Conclusiones:* En conclusión, la inmunoterapia frente a ácaros del polvo de casa no induce a una sensibilización a tropomiosina clínicamente relevante. En algunos casos, la alergia frente a ácaros y marisco tratada con IT puede mejorar los síntomas de la alergia alimentaria. Los niveles de IgE específica frente a Der p 10 y Pen a 1 pueden ser marcadores útiles para monitorizar a estos pacientes.

**Palabras clave:** Ácaro del polvo de casa. Marisco. Sensibilización. Inmunoterapia específica. Tropomiosina.

## Introduction

House dust mite (HDM) allergens are the leading environmental cause of respiratory allergies [1]. Subcutaneous immunotherapy (SCIT) using HDM extracts is an effective method of treating these allergies and has well-established clinical [2-7] and immunological effects [8-11]. However, owing to cross-reactivity between HDM allergens and allergens of other species and the number of potential routes of sensitization to those cross-reactive allergens, clinicians remain concerned about the safety of this treatment in certain cases.

HDM-sensitized patients can be classified according to their sensitization profiles into those sensitized only to the major allergens (group 1 and group 2 allergens of *Dermatophagoides* mites) and those with a broader pattern of sensitization, including highly cross-reactive allergens [12]. The most important cross-reactive allergen is tropomyosin (group 10 allergen of *Dermatophagoides*).

Allergenic tropomyosins are highly conserved proteins found in invertebrates such as arachnids (mites), insects (cockroaches), crustaceans (shrimps, lobsters, crabs), and mollusks (squids, snails) and are therefore considered panallergens [13]. The recognition of similar amino acid sequences by IgE in homologous tropomyosins is the basis of cross-reactivity between these phylogenetically distant species [14]. The prevalence of sensitization to tropomyosin among HDM-allergic patients varies greatly with geographical area. Generally, lower sensitization rates are found in European countries with a temperate climate (4% in Germany, 6-18% in Austria, 9% in northern France, 10% in Italy, and 18% in Sweden) [15-17], whereas higher rates are observed in subtropical/tropical regions (28% in southern France, 29% in Australia, and 55% in central Africa) [18-20]. This variability can be explained by exposure to different sensitizing allergens in different parts of the world. Airborne exposure to various mite and cockroach species and oral intake of crustaceans and mollusks can cause sensitization to tropomyosin [21]. The resulting IgE antibodies can cross-react with different tropomyosins, even those which did not induce their production [22].

Most of the currently available HDM extracts used for SCIT contain high concentrations of group 1 and 2 allergens, but may also contain low concentrations of other sensitizing molecules, including tropomyosin [23]. It remains unclear whether administration of allergen extracts during immunotherapy can induce clinically relevant sensitizations in patients previously sensitized to other allergens. Worsening of food allergy symptoms has been reported during HDM-SCIT [24,25] and attributed to new sensitization to tropomyosin [24]. Other studies were not able to confirm de novo sensitizations [26,27].

Patients sensitized to tropomyosin can develop a broad spectrum of allergic responses after ingestion of tropomyosin-containing seafood, ranging from mild oral allergy syndrome to severe, life-threatening anaphylactic reactions [21,28]. Therefore, it is important to identify HDM-allergic patients with a broader sensitization pattern, especially when planning SCIT. A tropomyosin from *Dermatophagoides pteronyssinus*

(Der p 10) is considered a useful diagnostic marker for identification [29].

The aim of this study was to assess whether HDM-SCIT induces new sensitizations to tropomyosin using rDer p 10 as a diagnostic marker. In sensitized patients, we also studied the dynamics of sIgE to tropomyosin from both mite (rDer p 10) and shrimp (rPen a 1), as well as clinical reactivity to tropomyosin-containing seafood during and after SCIT.

## Patients and Methods

### Patients

The study population consisted of 56 HDM-allergic patients (38 males) who had received a course of SCIT with HDM extracts. Their ages ranged from 14 to 48 years; other demographic characteristics are summarized in Table 1. Before SCIT, all patients had been diagnosed with persistent allergic rhinitis, and most had received treatment with oral H1 antihistamines, intranasal corticosteroids, or both. Patients with concomitant asthma had been treated according to the recommendations of the Global Initiative for Asthma (GINA). Some patients with concomitant atopic dermatitis had intermittently used topical corticosteroids. Allergy diagnoses were confirmed in all patients by positive skin prick test (SPT) results and determination of sIgE (fluorescent-enzyme immunoassay, class  $\geq 3$ ) to both *D pteronyssinus* and *Dermatophagoides farinae*.

Table 1. Demographic Characteristics of the Study Patients

Subjects, No.	56
Age, y, median (IQR)	24.5 (19.3-29.0)
Male sex, No. (%)	38 (67.9)
Clinical diagnosis, No. (%)	
Rhinitis	56 (100)
Conjunctivitis	35 (62.5)
Asthma	16 (28.6)
Atopic dermatitis	8 (14.3)

SCIT was performed with allergen extracts from *D pteronyssinus* and *D farinae* (Novo-Helisen Depot, Allergopharma Joachim Ganzer KG). The mite ratio in each extract was adjusted according to the respective sIgE levels in the serum of each patient. Immunotherapy was administered by subcutaneous injection according to the recommended dosing regimen. The details are shown in Table 2.

All patients read and signed the informed consent form approved by the local ethics committee (permission no. 01-1853, dated March 24, 2010).

### Measurement of sIgE to Tropomyosins

The levels of IgE antibodies specific for tropomyosin from *D pteronyssinus* and from brown shrimp (*Penaeus aztecus*) were measured using recombinant allergens (rDer p 10 and rPen a 1, respectively) using the CAP System fluorescent-enzyme immunoassay (Thermo Fisher Scientific - Phadia AB). The results were expressed in kU<sub>A</sub>/L.

**Table 2.** SCIT With Allergen Extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in a Group of 56 Patients

Ratio of <i>Dermatophagoides pteronyssinus</i> to <i>Dermatophagoides farinae</i> in the Allergen Extract		Duration of SCIT	
Ratio	Patients No. (%)	Years	Patients No. (%)
50:50	32 (57.1)	5.5	4 (7.1)
60:40	11 (19.6)	5	12 (21.4)
40:60	6 (10.7)	4.5	4 (7.1)
70:30	3 (5.4)	4	7 (12.5)
80:20	3 (5.4)	3.5	9 (16.1)
0:100	1 (1.8)	3	6 (10.7)
		< 3	14 (25.0)

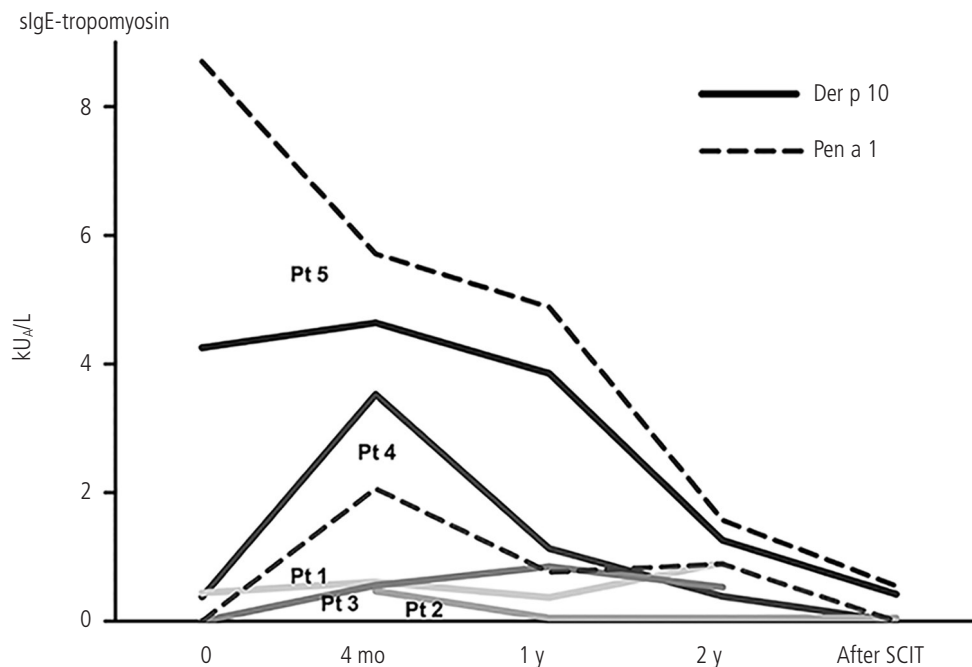
Abbreviations: SCIT, subcutaneous immunotherapy.

Initially, sIgE to Der p 10 was measured in each patient's serum before and after SCIT. If the treatment was discontinued before completion of a 3-year course or was ongoing at the time of this study, the initial measurements were taken in sera obtained before and 2 years after starting therapy. Additionally, sIgE to both Der p 10 and Pen a 1 was measured at 5 time points in all patients who displayed a positive result in the initial measurements: before initiation of treatment, after initial therapy (~4 months), 1 and 2 years after starting therapy, and after completion.

### Results

IgE antibodies specific for tropomyosin were found in only 5 out of 56 patients treated with HDM-SCIT (Figure). Of these, 3 patients had the antibodies before therapy.

Patient 1 had low levels of sIgE to Der p 10 at all time points (0.44 kU<sub>A</sub>/L prior to treatment, 0.61 kU<sub>A</sub>/L after 4 months of therapy, 0.37 kU<sub>A</sub>/L after 1 year, and 0.91 kU<sub>A</sub>/L after 2 years of therapy). In patient 2, a measurement was not possible before therapy, as no serum was obtained at that time point, but the level of sIgE to Der p 10 was low (0.42 kU<sub>A</sub>/L) after 4 months of therapy and disappeared at subsequent time points (1 and 2 years after starting therapy, and after completion). Patient 3 did not have antitropomyosin antibodies before SCIT, although low levels of sIgE to Der p 10 were detected during therapy (0.56 kU<sub>A</sub>/L after 4 months, 0.85 kU<sub>A</sub>/L after 1 year, and 0.53 kU<sub>A</sub>/L after 2 years). None of these patients had sIgE to Pen a 1.



**Figure.** Serum levels of IgE antibodies specific for tropomyosin from the house dust mite *Dermatophagoides pteronyssinus* (Der p 10) in 5 patients (solid lines) and for tropomyosin from the brown shrimp *Penaeus aztecus* (Pen a 1) in 2 patients (dashed lines) before, during (at 4 months and then at 1 and 2 years), and after subcutaneous immunotherapy with house dust mite extracts. Patients 1, 2, and 3 displayed only low levels of sIgE to Der p 10 (lighter solid lines), while patients 4 and 5 (apart from higher levels of sIgE to Der p 10) also displayed sIgE to Pen a 1 (darker solid and corresponding dashed lines, respectively). Pt indicates patient.

Patient 4 had a low level of sIgE to Der p 10 (0.38 kU<sub>A</sub>/L) and negative sIgE to Pen a 1 before SCIT. Following the initial phase of therapy, sIgE to Der p 10 increased (3.53 kU<sub>A</sub>/L), and sIgE to Pen a 1 was detected at a lower level (2.06 kU<sub>A</sub>/L) than sIgE to Der p 10. The levels of sIgE to both tropomyosins then gradually declined after 1 and 2 years, finally reaching negative values once SCIT had been completed.

Patient 5 had a relatively high level of sIgE to Der p 10 (4.25 kU<sub>A</sub>/L) and even higher level of sIgE to Pen a 1 (8.70 kU<sub>A</sub>/L) before SCIT. These levels gradually declined during SCIT to 0.42 kU<sub>A</sub>/L for Der p 10 and 0.55 kU<sub>A</sub>/L for Pen a 1 after completion.

In the remaining 51 patients treated with HDM-SCIT, antitropomyosin sIgE antibodies were not found either before therapy or at the last time point. IgE was determined after successful completion of SCIT in 39 patients; in 12 patients, whose treatment was discontinued earlier or was ongoing, IgE determinations were made after 2 years of therapy.

Clinically, only Patient 5 experienced symptoms of food allergy before starting immunotherapy. On several occasions, symptoms of oral allergy syndrome developed after ingestion of squid or shrimp. He was therefore advised to completely avoid those foods. After completion of SCIT, a food challenge was performed starting with a small piece of shrimp and then cautiously proceeding with larger quantities every 15 minutes until the complete meal (~150 g) was ingested. A similar food challenge was performed with squid. No clinical reactions occurred in either case. The patient was subsequently able to eat both squid and shrimp without symptoms.

## Discussion

SCIT with HDM extracts is an effective method of treating allergy. In our previous study based on the same study population [30], the efficacy of HDM-SCIT was clearly demonstrated during the treatment period through a gradual reduction in symptom severity and skin reactivity, a loss of allergen reactivity induced by nasal challenges, total IgE and sIgE dynamics, and increased production of sIgG4. In a subgroup of 39 patients, successful HDM-SCIT altered the expression of several T regulatory and FcεRI pathway genes; these changes in expression resembled the levels detected in the healthy population [31].

The observation that symptoms of food allergy to snail or shrimp can worsen in some patients undergoing HDM-SCIT [24,25] incriminated the tropomyosin present in HDM extracts as the culprit cross-reacting allergenic molecule [24]. However, most patients already displayed mild allergic reactions to tropomyosin-containing foods before starting immunotherapy, while a new sensitization was confirmed in only 1 patient, whose result was negative at baseline [24]. On the other hand, studies investigating the induction of new sensitization to tropomyosin during HDM immunotherapy showed a lack of such sensitizations [26,27]. Our results are consistent with those reported above, since none of the patients included developed clinically relevant sensitization to tropomyosin during the treatment period. In contrast with previous studies, we used a more specific approach to detect tropomyosin sensitizations that might result from exposure

to HDM allergens. We used mite tropomyosin (Der p 10) to screen our patients, while the 2 earlier studies used both fresh shrimp and commercial shrimp extract or shrimp tropomyosin (Pen a 1) [26,27]. Another advantage of our study is that we analyzed the dynamics of sIgE to both mite and shrimp tropomyosins during and after SCIT in all patients who initially screened positive.

Our approach revealed sIgE to mite tropomyosin in only 5 out of 56 patients treated with HDM-SCIT. Of those, 3 patients expressed low levels of sIgE to Der p 10 during the course of SCIT, while sIgE to Pen a 1 was not observed. Their sensitizations were not clinically relevant, since none of the 3 patients had allergic reactions after consuming tropomyosin-containing foods. The remaining 2 patients expressed sIgE to both tropomyosins, although with different dynamics in each case.

In one patient (Patient 4 [Results and Figure]), only sIgE to Der p 10 was found before SCIT. The level increased after the initial phase and then gradually decreased, finally reaching undetectable levels after completion of SCIT. Interestingly, sIgE to Pen a 1 was detected after the initial phase, but its level was lower than that of sIgE to Der p 10. Subsequently, it also decreased gradually to undetectable levels. The dynamics of sIgE to tropomyosin observed in this patient resemble the dynamics of IgE antibodies usually seen in SCIT patients. Therefore, knowing that this patient had a high level of sensitization to HDM before SCIT (the initial sIgE concentration for *D pteronyssinus* was 219.20 kU<sub>A</sub>/L), we concluded that his sensitization to tropomyosin resulted from exposure to HDM. The transitory appearance of sIgE antibodies cross-reacting with a homologous shrimp tropomyosin is probably SCIT-induced, although not clinically relevant. This conclusion is further supported by the fact that this patient had never experienced an allergic reaction after consuming tropomyosin-containing foods.

The other patient (Patient 5 [Results and Figure]) had expressed relatively high levels of sIgE to both tropomyosins before SCIT. The initial level of sIgE to Pen a 1 was twice as high as that of sIgE to Der p 10. Both levels then gradually declined during SCIT, before finally reaching a low range after therapy. The initial level of sensitization to mite in this patient (sIgE to *D pteronyssinus*, 55.50 kU<sub>A</sub>/L) was lower than in Patient 4, and he also displayed sIgE to brown shrimp. In addition to respiratory allergy, the patient had symptoms of oral allergy syndrome after consuming squid or shrimp before starting immunotherapy. The most surprising and unexpected finding was that the reduction in antitropomyosin sIgE levels observed during and after SCIT actually resulted in the complete loss of reactivity toward those foods, which was confirmed by oral challenge after the completion of SCIT. Even now, 4 years after therapy, he is still able to eat squid and shrimp without symptoms. Nevertheless, it is not entirely clear whether sensitization to tropomyosin in this patient was through ingestion of seafood, as would be suggested by the higher initial level of sIgE to Pen a 1, or whether it resulted from exposure to HDM, in which case it could be explained by the beneficial effect of HDM-SCIT.

Apart from not inducing new sensitizations, HDM-SCIT could have beneficial effects in patients with food allergy.

One study demonstrated a possible protection against new sensitizations to snail in children receiving HDM-SCIT [32]. A recently published report presented the case of a young man whose shrimp allergy improved from anaphylactic symptoms observed before to mild oral allergy syndrome after 1 year of HDM sublingual immunotherapy (SLIT) [33]. Furthermore, our results showed that the improvement achieved could persist for years after completion of HDM-SCIT. Earlier studies demonstrated that new sensitizations to tropomyosin did not occur regardless of whether patients were treated with mite SCIT or SLIT [26,27]. Both SCIT and SLIT might be effective in improving food allergy symptoms, since the patient from the aforementioned report was treated with SLIT [33], whereas the patient we describe received SCIT.

Food allergy to crustaceans or mollusks can worsen in patients receiving HDM-SCIT. Some adverse reactions could result from sensitizations to allergens other than tropomyosin [34] involved in cross-reactivity between seafood and mites and possibly induced by immunotherapy. Other reactions can be attributed to pre-existing sensitization to tropomyosin, which should be diagnosed before starting HDM-SCIT. Therefore, the presence of sIgE to Pen a 1 was shown to be a good predictor of clinical reactivity [35], whereas its levels correlated with the severity of clinical manifestations [34]. Accordingly, the patient with clinical reactivity toward seafood in our study experienced symptoms only initially, when the level of sIgE to Pen a 1 was relatively high. Unfortunately, the cutoff values that would enable us to predict the severity of the reaction have not yet been established. However, our results show that besides measuring sIgE levels to both tropomyosins when HDM-SCIT is considered in patients with seafood allergy, it may also be useful to monitor the dynamics of sIgE during and after the protocol. Such monitoring could guide diagnostic and therapeutic decisions.

In conclusion, our results confirm that induction of clinically relevant sensitization to tropomyosin is a highly unlikely consequence of HDM-SCIT. Furthermore, in patients with combined mite and seafood allergy, HDM-SCIT can actually improve food allergy symptoms, at least in some cases. Since the risk of adverse reactions to seafood needs to be closely monitored, levels of sIgE to both Der p 10 and Pen a 1 may be useful markers. Additional studies involving a larger number of similar patients would consolidate our results.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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### Previous Presentation

Data from this manuscript were presented at the Food Allergy and Anaphylaxis Meeting, FAAM, 2013 (February 7-9, 2013 in Nice, France) as a poster entitled "House dust mite-specific immunotherapy and tropomyosin sensitizations: harm or benefit for patients?" (Reference no. PS03-044).

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