

ANALYSIS OF EXPRESSION AND AMINO ACID SEQUENCE OF THE ALLERGEN MAG 3 IN TWO SPECIES OF HOUSE DUST MITES – *DERMATOPHAGOIDES FARINAE* AND *D. PTERONYSSINUS* (ACARI: ASTIGMATA: PYROGLYPHIDAE)

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Asman M, Solarz K, Szilman E, Szilman P: Analysis of expression and amino acid sequence of the allergen Mag 3 in two species of house dust mites – *Dermatophagoides farinae* and *D. pteronyssinus* (Acari: Astigmata: Pyroglyphidae). *Ann Agric Environ Med* 2010, **17**, 45–48.

Abstract: In the 90s of the XX century, 2 new and important allergens of house dust mites were cloned and sequenced: Mag 1 and Mag 3. However, the second allergen has been identified to date only in extracts of *Dermatophagoides farinae* [DF]. In this work, we aimed to detect expression of this important allergen and for the first time analyze to the amino acid sequence in other species of house dust mite – *Dermatophagoides pteronyssinus* [DP]. We were able to confirm the expression of allergen Mag 3 in DF and to exclude it in DP. By sequencing the products of DNA amplification, we revealed the nucleotide sequence encoding allergen Mag 3 in DF. This analysis enabled detection of 9 single base changes. An analysis of encoded amino acid sequence by triplets with substituted nucleotides revealed that 8 changes were polymorphic, and 1 was a mutation substituting GTG (valine) for ATG (methionine) at 236 position. However, the presence of amino acid sequence difference in this allergen might suggest that there exist other isoforms which can make difficult both diagnosis as well as immunotherapy in persons who produce allergic response to this allergen. The variants of allergen Mag 3 (group 14) are still not known beside the very good known allergen variants of the other main groups 1, 2, 4, 5 or 7. Thus, the identification and definition of allergic properties of allergen Mag 3 variants needs to be further investigated.

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Key words: allergy, house dust mites, house-dust-mite allergens, Mag 3, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*.

INTRODUCTION

Allergic diseases affect about 40% of the human population. The most frequent allergic diseases caused by aero-allergens are rhinitis, conjunctivitis, asthma or atopic dermatitis [3, 16]. House dust mites classified in the family Pyroglyphidae (Acari: Acaridida) are recognized as the most important risk factors causing indoor allergies [3, 4]. Three mite species, *Dermatophagoides pteronyssinus*, *D. farinae* and *Euroglyphus maynei*, are the most abundant in house dust throughout the world [2, 6]. Also, they are the major sources of indoor inhalant allergens, causing both the sensitization of atopic subjects and asthmatic attacks

in patients [12, 13, 14, 17]. Localization of the allergens in mites varies; they may be found both in bodies as well as in excuviae, secretion (saliva) and excretions (faecal pellets). The major allergens responsible for the allergic reactions are the allergens classified in groups 1 and 2 [2, 3, 4]. An equally important group of allergens is group 14. These are proteins homologous with apolipoprotein with large molecular weight from 177–190 kDa [3]. To this group belongs allergen Mag 3 cloned and sequenced in the 90s of the XX century, and Mag 1 which was detected in *D. farinae*, *D. pteronyssinus*, *E. maynei* and *Blomia tropicalis* [1, 5, 7, 8]. To date, the Mag 3 was discovered only in extracts of *D. farinae*. However, its precise localization is

not clear. It is probably related to the somatic parts of the mite body and does not appear in the mite faeces. The Mag 3 does not cross-react with anti-Der f 1 and anti-Der f 2, and its allergenicity was demonstrated by *in vivo* allergic tests and the histamine trials. In this study, we have examined the expression of Mag 3 in another species of house dust mite, namely *D. pteronyssinus*. We also examined the amino acid sequence of the protein which seems to be the most important allergen in humans [7].

MATERIALS AND METHODS

Material for the research were frozen extracts from the mites bodies *D. farinae* and *D. pteronyssinus*. The mites were obtained from a breeding culture at the Department of Parasitology of the Silesian Medical University in Katowice. Each homogenate was made from about 1,000 mite bodies and suspended in 500 µl one time concentrated solution SB (Sample Buffer). Three homogenates were made for each species. From the extracts poly(A)-mRNA was isolated with the use of mRNA isolation kit (Quiagen, Germany). The concentration of total isolated RNA was determined spectrophotometrically. A cDNA was obtained by 2 steps of RT-PCR reactions using commercial kit (Sigma Aldrich, Germany).

The components for PCR amplification were as follows: about 200 ng cDNA, 1 µl Oligonucleotide Mix (Roche, Germany), 5 µl Buffer + Mg (Roche, Germany), 4 µl of mixture of 2 primers specific for Mag 3 (2 µl of the forward primer and 2 µl of the reverse primer) and 0.4 µl Fast Start Taq Polymerase (Roche, Germany). The final volume of 50 µl was adjusted using pure sterile deionized RNA-ase free water. Samples were initially denatured for 240 sec at 95°C. Subsequent cycles were at 95°C for 30 sec (denaturation), 48.5°C for 30 sec for first pair specific primers, and 58.1°C for 30 sec for second pair specific primer (annealing), and 72°C for 19 sec for first pair specific primers and 72°C for 23 sec for second pair specific primer (elongation). Thirty four cycles were performed. The final extension were in 72°C for 420 sec. PCR products were visualized with UV light following separation in 0.8% agarose gel containing ethidium bromide. The PCR products were of expected size of about 420 bp and 520 bp, respectively. The DNA fragments of correct size were recovered from the agarose gel using the DNA isolation kit (EURX, Germany) and about 200 ng of cDNA was purified with alkaline phosphatase and endonuclease (SAP-EXO) to remove excess of primers and free oligonucleotides (dNTP's).

For DNA sequencing, the purified template cDNA fragments were mixed with each primer separately. The sequencing PCR was conducted according to the procedure supplied by Applied Biosystems. Briefly, the sequencing PCR products were purified by ethyl alcohol precipitation and the DNA was solubilized in 20 µl of MegaBase (Applied Biosystems, USA). The DNA sequences were analyzed using the capillary DNA analyzer ABI PRISM 310

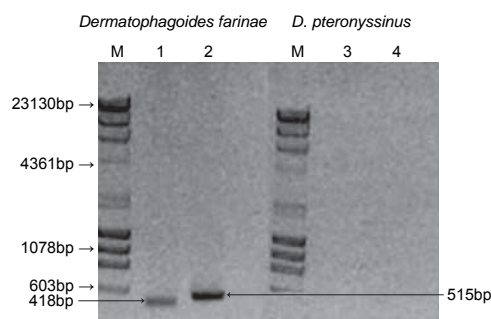


Figure 1. PCR amplification fragments of allergen Mag 3 in *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. M – marker height (Finnzymes, Finland); 1 and 3 – Mag 3 fragments height 418 bp; 2 and 4 – Mag 3 fragments height 515bp.

(Applied Biosystems, USA). For confirmation of the results, data from 3 independent homogenates were analysed for each species.

RESULTS

By PCR and DNA sequencing, an expression of allergen Mag 3 in *D. farinae* but not in the second studied species, *D. pteronyssinus* was detected (Fig. 1). Analysis of the DNA sequences revealed the presence of 9 single nitrogen base changes.

Subsequent amino acid sequence analysis revealed that 8 of these changes were polymorphisms; however, one change proved to be a mutation. The mutated sequence was compared with a reference sequence deposited at the GeneBank with the accession ID: D17686.1 (Tab. 1, Fig. 2). These results were confirmed by 3 independent analyses.

DISCUSSION

The house-dust-mite allergens Mag 1, Mag 3 and the high-molecular-weight protein M-177 have been identified as parts of the apolipoprotein-like allergen and belong to the 14 group. Apolipoproteins are hydrophobic proteins found in lipid bodies and lipid transport particles of insects.

Table 1. Single nucleotide changes in the sequence of DNA encoding Mag 3 of *Dermatophagoides farinae*.

No.	Nucleotide position	Amino acid change	Note
1.	G236A	Val-Met	M
2.	G409A	Gly-Gly	P
3.	A448C	Pro-Pro	P
4.	C697T	Thr-Thr	P
5.	A706G	Glu-Glu	P
6.	G739A	Ser-Ser	P
7.	T803C	Gly-Gly	P
8.	A808C	Ala-Ala	P
9.	T826C	His-His	P

M – change resulting in mutation, P – polymorphic change

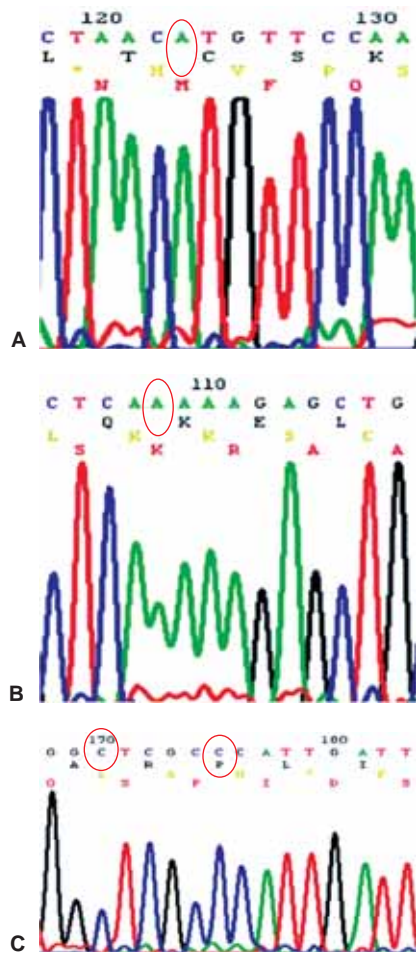


Figure 2. Representative changes found in a nucleotide sequence of DNA encoding Mag 3 in *Dermatophagoides farinae*. A – change with a mutation character, position G236A; B – change with a polymorphic character, position G739A; C – changes with a polymorphic characters, positions T803C and A808C.

They have regions of identity to human apolipoprotein and to lesser degree to the vitellogenins [5]. This allergen was previously described in *D. farinae* as Mag 1 (Der f 14), in *Euroglyphus maynei* as a high-molecular-weight M-177 (Eur m 14, rEur m 14) and, moreover, in *D. pteronyssinus* (Der p. 14) and *Blomia tropicalis* (Blo t 14, Blo t Mag 1). A homologue of Der f 14 and Eur m 14 has also been identified recently. This allergen is now known to be a hemolymph apolipoprotein [5, 9, 18]. The second protein Mag 3 is probably triggered through somatic parts of the mite body, such as interstitial tissue of the esophagus, gut, and other internal organs. It is also not related to digestive enzymes, as many of other mite allergens are. This allergen is also not detected in mite faecal pellets, too. Its direct localization in the mite body is unknown [8]. The sequences of both proteins, Mag 1 and Mag 3, are different. The allergen Mag 3 does not give cross-reactions with anti-Der f 1 and anti-Der f 2, and its allergenic potential was demonstrated through *in vivo* allergic tests and histamine trials. These results suggest that this is a new kind of the high molecular

weight allergen [8]. The protein Mag 3 also shows homology to apolipoprotein, thus sharing structural homology also with vitellogenin, a protein found in egg yolk, and causes allergic reaction in over 70% of patients. Two fragments of Mag 3 have molecular weight of about 80–83 kDa and 95–101 kDa, respectively [3]. This allergen so far has been identified only in *D. farinae*. Our results also confirmed the previous findings. An expression of this allergen has not been studied in other pyroglyphid dust-mite species to date moreover, the role of this allergen is still under discussion. Some researchers classify it as an additional antigen, others, however, propose giving it the role of a major allergen. Fuji *et al.* [7] reported that the lymphocytes that respond to mites antigens are the T-cells. Thus, they postulate that in order to study the specific immunotherapy against the major mites allergens, the key is to identify the mechanism of the T-cells activation and determine their epitopes. It has been also shown that the Mag 3 has the potential ability to stimulate proliferation of T-lymphocytes, which play a part in the immunological response to allergic reactions that are triggered by mites, and may be a potential candidate for protein vaccine [7].

The human IgE-antibodies have an affinity to the allergen Mag 3 comparable with their affinity to allergen Der f 2, which is the major allergen in *D. farinae* [7, 8]. Also, a similar frequency of sensitization and the potential application of this allergen in the immunotherapy allows the conclusion that this poorly-known allergen should be recognized as the one of the most important allergens in this species.

Until now, assays identifying variants Mag 3 have not been available. Only 1 sequence is known which is available in the GeneBank under ID: D17686.1. Results presented here permit the finding that Mag 3 is similar to the allergen Gly d 2 or Lep d 2 which also have various isoforms. This polymorphism may influence the modification of specific T-lymphocyte response due to the difference in the amino acid sequence that may cross the competence of MHC molecules, which are presented on the cells [10]. However, there are few studies on environmental polymorphisms of the main allergens of *D. pteronyssinus* and none for *D. farinae* [15]. The allergen polymorphism may have influence on the recognition by T-lymphocytes, monoclonal antibodies, and IgE class antibodies of allergic patients. It may have influence on the basal level of allergenic potential, as well as on cross-reactivity with other allergens [11].

Information on the allergen sequence is important because it provides the data for optimal design of the allergen. It permits use of this allergen in genetic engineering and precise structure-function analyses of the major mite allergens [10]. The use of various allergen isoforms has also been suggested as an alternative to the conventional immunotherapy for allergic diseases [11]. Thus, the definition of allergic properties of allergen Mag 3 variants needs to be better understood.

Comparison of the expression of house dust mite allergen Mag 3 in *D. farinae* and *D. pteronyssinus* led us to

conclude that it appears only in this first species. However, the single nucleotide changes affecting an amino acid sequence of this allergen may suggest the existence of numerous isoforms, which can make difficult both diagnosis as well as immunotherapy in persons who produce allergic reaction to this allergen [1, 5, 7, 8, 15].

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