

Sequence polymorphisms of Der f 1, Der p 1, Der f 2 and Der p 2 from Korean house dust mite isolates

Kyoung Yong Jeong · In-Yong Lee · Tai-Soon Yong · Jae-Hyun Lee · Eun-Jin Kim · Joo-Shil Lee · Chein-Soo Hong · Jung-Won Park

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Abstract Amino acid sequence variations have possible influences on the allergenicity of allergens and may be important factors in allergen standardization. This study was undertaken to investigate the sequence polymorphisms of group 1 and 2 allergens from Korean isolates of the house dust mites *Dermatophagoides farinae* and *D. pteronyssinus*. cDNA sequences encoding group 1 and 2 allergens were amplified by RT-PCR and compared the deduced amino acid sequences. Der f 1.0101, which appeared in 64.0 % of the 50 sequences analyzed, was found to be predominant. Among the Der p 1 sequences, Der p 1.0102 and 1.0105 were predominant (58 %). Among the Der f 2 sequences, Der f 2.0102 (40.7 %) and a new variant with Gly at position 42 (27.8 %) were predominant. The deduced amino acid sequences of 60 Der p 2 clones were examined, and 28 variants with 1–5 amino acid substitutions were found. Interestingly, all of the Der p 2 sequences had Thr instead of Lys at position 49. Two variants (Leu40, Thr49, and Asn114 (26.6 %); Val40, Thr49, and Asn114 (20.0 %)) were found to be the most predominant forms of Der p 2. Der p 1 has a high rate of sporadic substitutions and the group 2 allergens show a more regular pattern with orderly associations of amino acid substitutions. Der f 1 and Der p 2 from Korean mite isolates have unique amino acid sequence polymorphisms. These findings provide important data for house dust mite allergen standardization.

Keywords Allergens · Der f 1 · Der f 2 · Der p 1 · Der p 2 · Polymorphism

K. Y. Jeong · J.-H. Lee · C.-S. Hong · J.-W. Park (✉)
Department of Internal Medicine and Institute of Allergy, Yonsei University College of Medicine,
50 Yonei-ro, Seodaemun-gu, Seoul 120-752, Korea
e-mail: parkjw@yuhs.ac

K. Y. Jeong
e-mail: jeongky@yuhs.ac

I.-Y. Lee · T.-S. Yong
Department of Environmental Medical Biology and Institute of Tropical Medicine,
Arthropods of Medical Importance Resource Bank, Yonsei University College of Medicine,
Seoul 120-752, Korea

E.-J. Kim · J.-S. Lee
Center for Immunology and Pathology, Korea National Institute of Health, Osong, Korea

Introduction

House dust mites are the most important causes of respiratory allergic disorders (Jeong et al. 2007). Among house dust mite allergens, group 1 allergens (Der f 1 and Der p 1) and group 2 allergens (Der f 2 and Der p 2) dominate overall allergic responses (Thomas et al. 2007). Consequently, group 1 and 2 allergens are of special interest in the preparation and standardization of house dust mite allergen extracts.

Variants of group 1 and 2 allergens have been described from different geographical regions (Piboonpocanun et al. 2006). In particular, amino acid sequence variations of Der p 2 variants were shown to have an influence on IgE binding reactivity that differed by up to 50 % (Park et al. 2002). It has also been reported that even a single amino acid substitution in Der p 2 can induce different cytokine production of T cells (Hales et al. 2002). Therefore, identification of the representative variants in an environment of interest is essential for the accurate characterization of allergens.

The standardization of allergen extracts is important for the diagnosis and treatment of allergic diseases (Jeong et al. 2011). The allergenicity of allergen extracts is known to correlate with the amount of major allergen in the extract (Dreborg and Einarsson 1992; van Ree 2007), and determination of the major allergen concentration generally relies on immunoassay kits using monoclonal antibodies. However, sequence polymorphisms of allergens influence the reactivity of monoclonal antibodies. Mite group 2 allergens are also known to have an influence on allergenicity and quantification (Park et al. 2002). Recently, a higher concentration of Der f 1 was detected in dust samples using antibodies locally produced in Thailand compared to a commercially available immunoassay kit (Indoor Biotechnology, UK) (Sookrung et al. 2011). Therefore, polymorphisms of the major allergens are thought to be one of the main problems in the standardization of allergen extracts.

In this study, we investigated the sequence polymorphisms of major allergens from two dominant species of house dust mites, *Dermatophagoides farinae* and *D. pteronyssinus*, in Korea (Ree et al. 1997). An examination of the variants of these two species will provide important data for house dust mite allergen standardization.

Methods

House dust mites

A colony of *D. farinae* was isolated from beds in Bukgajwa-dong, Seoul and has been maintained in the Department of Environmental Medical Biology and Institute of Tropical Medicine, Arthropods of Medical Importance Resource Bank, Yonsei University College of Medicine, Seoul, Korea, since 1999. A *D. pteronyssinus* colony was kindly provided by Dr. Jihyun Kim, Department of Pediatrics, Samsung Medical Center, Seoul, Korea. Mites were cultured in the insect-rearing facilities in the Arthropods of Medical Importance Resource Bank with a diet consisting of a mixture of fish food powder and dried yeast, as described previously (Ree et al. 1997). Mites were harvested from culture media by using sieves (600 and 75 µm opening) and a saturated salt solution (Ree et al. 1997). Harvested mites were kept at −80 °C until use.

Sequence analysis of Der f 1, Der p 1, Der f 2, and Der p 2

Reverse transcriptase-PCR was performed to amplify cDNA fragments of Der f 1, Der p 1, Der f 2 and Der p 2. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad,

CA, USA) from about 100 mg of mites for each preparation and first-strand cDNA was synthesized using 10 µg of total RNA as templates. Six independent PCR reactions were carried out for each allergen. The sequences of oligonucleotides used in the PCR reactions are listed in Table 1. All forward primers are located at the upstream regions of mature allergens. Reverse primers of group 1 allergens and group 2 allergens covers the last 13 and 19 bases, respectively. ExTaq DNA polymerase (Takara, Tokyo, Japan), which has proofreading activity, was employed for accuracy. The PCR products were ligated into the pCR4 TOPO vector (Invitrogen) and sequences were determined in both directions at Solgent (Daejeon, Korea) from 4 to 14 clones of each PCR reaction. DNA sequences were analyzed and aligned using the CLUSTAL X program (<ftp://ftp-igbmc.u-strasbg.fr/pub/ClustaX>) (Thompson et al. 1997). For comparison, all cDNA sequences were translated into amino acid sequences.

Results

Der f 1 polymorphism

A total of 50 Der f 1 cDNA sequences were analyzed and 19 variants were identified (Fig. 1). Der f 1.0101 was found to be predominant (32/50, 64.0 %). All of the variants showed only 1 or 2 amino acid substitutions (99.1–99.6 % identities). The most frequently occurring variant, Der f 1.0101, was submitted to the GenBank database under accession No. JN222804.

Der p 1 polymorphism

Fifty cDNA clones encoding Der p 1 sequences were analyzed and 19 variants were found (Fig. 2). All of the variants showed one to three amino acid substitutions (98.6–99.5 % identities). Der p 1.0102 (22/50, 44.0 %) was the most commonly found variant, followed by a variant (Der p 1.0105) with Ala124 and Gly182. A new variant with Ala124 and Ser182 (4/50, 8.0 %) was deposited in the GenBank database under accession No. JN222803.

Der f 2 polymorphism

A total of 17 variants were identified by the analysis of 54 clones encoding Der f 2 sequences (Fig. 3). Der f 2.0102 (22/54, 40.7 %) and a new variant which has Gly at

Table 1 Sequences of oligonucleotides used for RT-PCR

Oligonucleotides	Sequences
Dp1F	5'-GTCCTCTCGAGAAAAGAGAGGCTCGTCCATCATCGATCAAAAC-3'
Dp1R	5'-GTGGTGCGGCCGCTTAGAGAATGACAACATATGGATATTC-3'
Df1F	5'-GTCCTCTCGAGAAAAGAGAGGCTCGTCCAGCTTCAATCAAAATC-3'
Df1R	5'-GTGGTGCGGCCGCTTCACATGATTACAACATATGGATATTG-3'
Dp2F	5'-CGCATATGGAATTCGATCAAGTCGATGTCAAAGATTG-3'
Dp2R	5'-CAGGTCGACATCGCGATTTTAGCATGAGTAG-3'
Df2F	5'-CGCATATGGAATTCGATCAAGTCGATGTAAAGATTGTG-3'
Df2R	5'-CAGGTCGACATCACGGATTTTACCATGGGTAG-3'

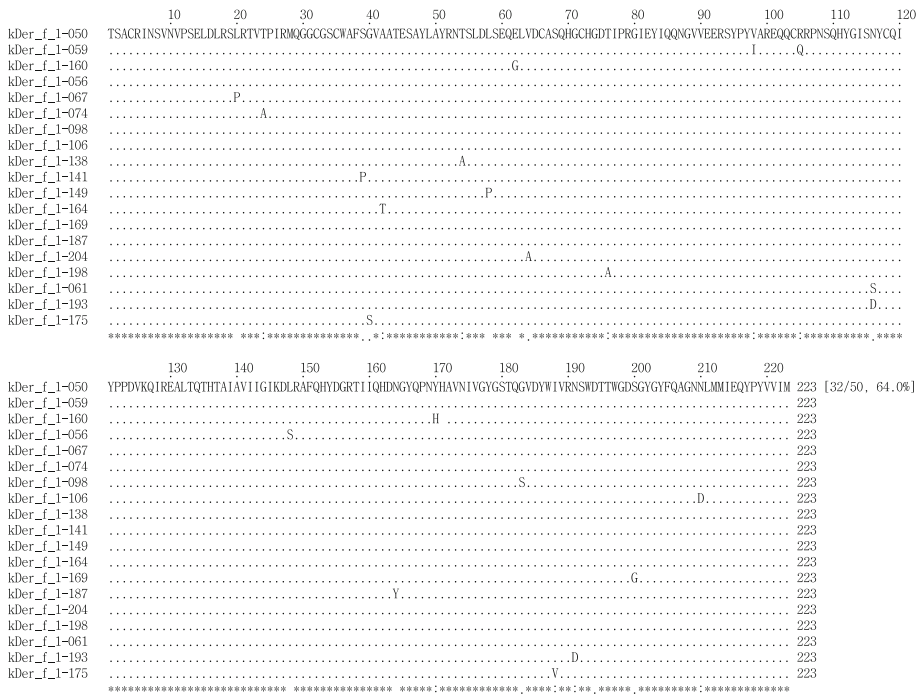


Fig. 1 Sequence alignment of Der f 1 variants from Korean *Dermatophagoides farinae* isolate. The percentage and frequency of each variant is shown in parentheses. Asterisk, identical; colon, highly conserved; dot, less conserved

position 42 (15/54, 27.8 %) were found to be the predominant variants. Der f 2.0116 was also identified (3/54, 5.6 %). A new variant with Gly42 was deposited in the GenBank database under accession No. JQ698663.

Der p 2 polymorphism

Deduced amino acid sequences of 60 Der p 2 clones were examined and 28 variants were found (Fig. 4). Interestingly, all the sequences from the Korean isolate had Thr at position 49, whereas most of the Der p 2 variants identified to date (Der p 2.0101–2.0114) have Lys at this residue (<http://www.allergen.org>). Only one reported variant, Der p 2.0115, had an amino acid substitution of Lys to Thr at position 49. A variant with Leu40 and Thr49 was the most frequently encountered (16/60, 26.7 %), followed by a variant with Val40 and Thr49 (12/60, 20.0 %). Three variants with an amino acid substitution at Met110, substitutions at Thr26, Val40, Thr48, Met110 and Glu113, and substitutions at Val40, Thr48, Met110 and Glu113, compared to the dominant form, were also found (3/60, 3/60, 3/60, respectively). These five new variants were submitted to GenBank under accession No. JN222805-9.

Discussion

The content of individual major allergens is known to correlate with the biological potency and IgE reactivity of allergen extracts, which are widely used for the diagnosis and

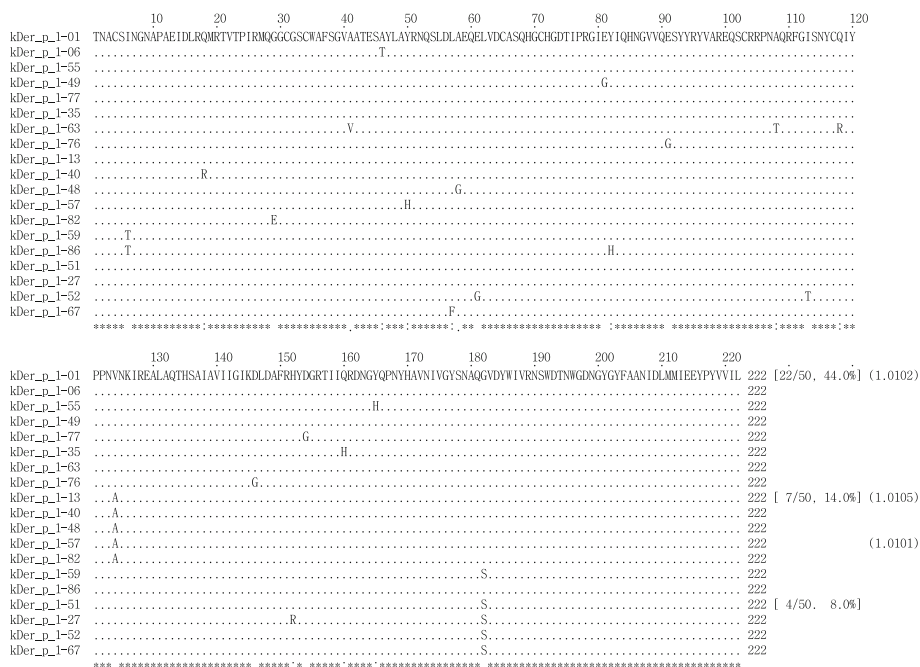


Fig. 2 Sequence variations of amino acids in Der p 1 from Korean *Dermatophagoides pteronyssinus* isolate. The percentage and frequency of each variant is shown in parentheses. Variant numbers given by the Allergen Nomenclature Subcommittee are provided at the end of the sequence

management of allergic disorders (Dreborg and Einarsson 1992). Much effort has been made to facilitate direct comparison of allergen extracts produced by various manufacturers (van Ree et al. 2005). However, amino acid sequence variations in major allergens are known to affect B cell and T cell immunogenicity. Different variants of house dust mites in different geographical regions have been reported and these differences should be considered in the diagnosis and management of allergic patients, as well as in the planning and interpretation of epidemiologic studies. Therefore, it is important to investigate the sequence polymorphisms of major allergens for the standardization of allergen extracts reflecting differences in regional environments.

A single variant of Der f 1, Der f 1.0101 constituted 64 % (32/50) of the clones analyzed, and could be representative of Korean Der f 1. It was described that the frequency of polymorphism in the Der f 1 of Thai mites was much less than that found for Der p 1 (Piboonpocanun et al. 2006). However, this does not seem to be the case in our Korean isolates. The other 18 variants identified in this study showed only sporadic substitutions of one or two amino acids.

For Der p 1, variants Der p 1.0102 and Der p 1.0105 were found to be the most representative variants in the Korean isolate. Substitution of an amino acid at position 124 (Val or Ala) is known to be important in human T cell responses (Smith et al. 2001a, b). Another substitution at position 182 (Ser or Gly) was also found to be polymorphic, and other substitutions were sporadic. This substitution at position 182 (Ser) (16 %, 8/50 clones) has not been observed elsewhere, but occurred at a high frequency even compared to the only frequent substitution at position 124 (22 %, 11/50 clones) found in other studies. Seven of 8 clones with substitution at position 124 have varying substitutions in

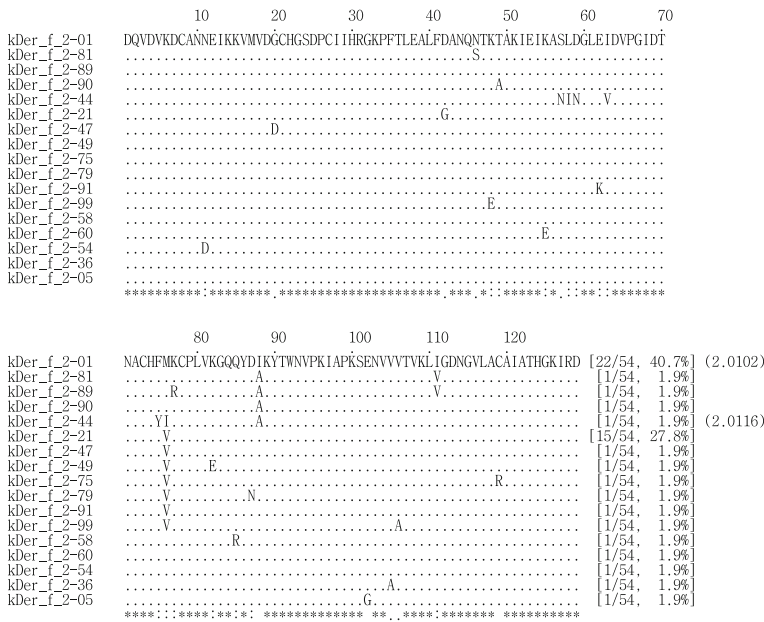


Fig. 3 Sequence alignment of Der f 2 variants from Korean *Dermatophagoides farinae* isolate. The percentage and frequency of each variant is shown in parentheses. Variant numbers given by the Allergen Nomenclature Subcommittee are provided at the end of the sequence

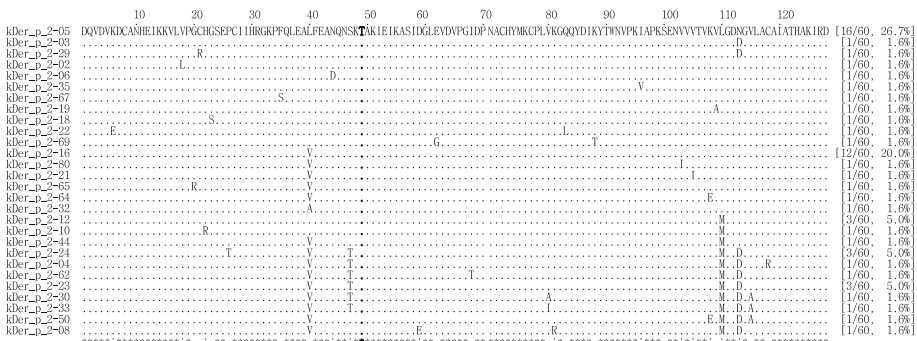


Fig. 4 Sequence variations of amino acids in Der p 2 variants from Korean *Dermatophagoides pteronyssinus* isolate. The percentage and frequency of each variant is shown in parentheses. Variant numbers given by the Allergen Nomenclature Subcommittee are provided at the end of the sequence. Unique amino acid substitutions in the Korean isolate are shown in bold

other positions. A mixture of Der p 1.0102, Der p 1.0105 and a new variant with Ser at position 182, which constituted 66 % (33/50) of the clones investigated, may be a good representation of Korean Der p 1.

Der f 2.0102 and a new variant with Gly at position 42 were found to be the most common Der f 2 variants. Interestingly, only a single variant (kDer_f_2-44 in Fig. 3) had Der f 2.0117-like motif NIN at position 57–59 (along with 63 V and 75Y), which is known to be the most frequent type of sequences from Thailand and Europe (Piboonpocanun et al. 2006). This difference suggests that there may be a big difference in the Korean population.

In a previous study, we identified a Der f 2 variant with Glu at position 100 (Jeong et al. 2002). Monoclonal antibodies produced against a recombinant Der f 2 variant with Glu100 were shown to be sensitive for detection from dust samples. However, this variant was not identified in this study. This may be a product of PCR error because Taq DNA polymerase, which does not have proofreading activity, was employed and DNA sequences of only 15 clones were analyzed from a PCR product of only one reaction in the previous study. In the present study, ExTaq DNA polymerase, which has proofreading activity, was employed for RT-PCR and at least 50 clones (54 clones for Der f 2) were examined from six independent PCR reactions. Still, there are some differences between house dust mites collected from different environments and house dust mites colonized and maintained in a laboratory. Sensitive detection of Der f 2 from dust samples in a previous study was achieved using monoclonal antibodies produced against a recombinant Der f 2 variant with Glu at position 100, probably due to the recognition of nonpolymorphic amino acids. A mixture of Der f 2.0101, Der f 2.0102 and Der f 2.0116, which constituted 74.1 % (40/54) of the clones examined, may be the best representation of Der f 2 in Korea.

All of the Der p 2 sequences analyzed were found to have Thr at position 49, whereas most of the variants identified to date have Lys (Der p 2.0101–0115) or Asn (Der p 2.0113). Notably, most of the variants in this study had Ser at position 47 (83.3 %, 50/60), which is known to have a higher IgE binding affinity, and Asn at position 114 (76.7 %, 46/60), which is responsible for strong binding to the monoclonal antibodies, 1D8 and 4G7. This variation is consistent with a previous study on Der p 2 from Korean house dust mites (Jin et al. 2003). Two variants constituted 46.6 % (28/60) of Der p 2 variants from Korea. However, it may be necessary to include three variants that occurred three times each (61.7 %, 37/60) for an accurate representation of Der p 2. Group 2 allergens tend to follow orderly pattern where different substitutions occur together although they have frequent polymorphisms.

Many studies have examined the influence of sequence polymorphisms of mite group 1 and 2 allergens on antibody (human IgE or mouse IgG) binding (Park et al. 2002; Smith and Chapman 1997; Hakkaart et al. 1998; Smith et al. 2001a, b; Mueller et al. 2001; Ichikawa et al. 2005; Takai et al. 2005; Chruszcz et al. 2009). Most of the substitutions found in this study were not in the epitope regions of the monoclonal antibodies, except Der p 2. However, amino acid substitutions not in the epitope regions may also have an effect on antigenicity by slightly altering the secondary and tertiary structures and subsequently affecting B cell interaction (Lombardero et al. 1990). The influence of these sequence variations on IgE binding and T cell reactivity has yet to be investigated.

New variants of major allergens from Korean house dust mite isolates were identified in this study. These variations may be the cause of significant differences in the determination of mite group 1 and 2 allergens measured by commercial two-site ELISA. It may be necessary to produce a regional standard using a mixture of three to five variants for monoclonal antibody-based quantification. The information obtained in the present study could be useful for the development of effective diagnostic and immunotherapeutic reagents.

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