

# Identification of a Novel Cat Allergen – Cystatin

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## Key Words

Allergen cat · Cystatin · Cysteine protease inhibitor · Asthma

## Abstract

**Background:** Cat allergen is an important cause of sensitization among children with asthma in Japan. Although there is good evidence that cats produce other allergens, only one major allergen, Fel d 1, has been studied in detail. **Aims:** To identify and define the molecular structure of the other potential cat allergens. **Methods:** A cat skin cDNA library was screened using IgE antibodies to cat dander and selected clones were sequenced and expressed. **Results:** One cDNA clone contained an open reading frame encoding a 98-amino acid residue protein. Sequence homology searches revealed a high degree of identity with bovine and human cystatin A, 79 and 75%, respectively. This cat cystatin clone contained the conserved cysteine protease motif and two of three lipocalin motifs. By plaque immunoassay, 60–90% of cat allergic sera had IgE Ab to cat cystatin. This cysteine protease inhibitor motif was partially conserved in dog allergens, Can f 1 and Can f 2, which are lipocalins. Recombinant cystatin was produced in *Escherichia coli* cells and purified as an 11-kD protein, corresponding to the predicted MW of cystatin. The structure of cat cystatin was modeled on human cystatin B using the SWISS-MODEL. **Conclusion:** A newly identified allergen, cystatin, has been cloned from cat skin and is a member of the cysteine protease inhibitor family.

## Introduction

Dander from domestic animals, notably cats, is an important cause of asthma and rhinitis among children with asthma in Japan [1]. The major cat allergen, Fel d 1, has been extensively characterized and the nucleotide and deduced amino acid sequence of Fel d 1 were obtained with PCR techniques [2]. Fel d 1 is produced in skin sebaceous glands and salivary glands [3, 4]. Fel d 1 is a 17-kD glycoprotein composed of two chains (chain 1 and chain 2) that is secreted as a 35- to 39-kD homodimer. Fel d 1 chain 1 shows limited sequence homology to rabbit uteroglobin and chain 2 has an N-linked glycosylation site [2].

Fel d 1 has been considered as an excellent marker of the immune response to cat allergens and has been used to monitor cat allergen in house dust and airborne cat allergen. However, absorption of Fel d 1 cannot remove allergenic activity of cat dander extract completely and several other allergens, including cat albumin, have been identified in cat dander extract [5, 6]. In this study, we screened a cat skin cDNA expression library with pooled IgE antibodies from cat-allergic patients to identify other potential cat allergens.

## Materials and Methods

Five sera from cat-allergic patients were pooled and used for screening of a cat skin cDNA expression library. Thirty-six cat-allergic sera (cat dander CAP class: 3–6) were used to further screen positive clones by plaque immunoassay. The cat skin cDNA library was prepared in the Uni-ZAP expression vector (Stratgene, La Jolla, Calif.) and screened with pooled human IgE antibodies. Selected cDNA

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clones were reassessed for IgE antibody binding by plaque immunoassay using individual sera.

The cDNA was isolated and sequenced using an ABI Prism 1000 Model 377 automated DNA sequencer. Sequences were analyzed using the GCG sequence analysis package. Protein sequences were compared with the PIR, SwissProt, Genbank, EMBL, EST, GSS databases using FASTA and BLAST. Selected cDNAs were ligated into the pET-22b(+) vector. Recombinant allergen was purified from the periplasmic space and purity was analyzed by silver-stained SDS-PAGE. The structure of cat cystatin was modeled using the SWISS-MODEL computer package.

## Results

A cat skin cDNA library was screened using pooled IgE antibodies to cat skin. Three positive clones (A, B and C) were identified and sequenced. Nucleotide sequencing showed that clone A and C contained an open reading frame, coding for a 98-amino acid residue protein (MW ~ 11 kD). The proteins encoded by clones A/C and clone B were assessed using a panel of sera from cat-allergic patients by plaque immunoassay. The stronger intensity of IgE antibody binding was observed using protein encoded by A/C rather than clone B. 60–90% and 25% of sera from cat-allergic patients gave positive IgE antibody plaques to the protein encoded by clone A/C or clone B, respectively.

Sequence similarity searches showed that clone A/C showed homology with bovine and human cystatin A, 79 and 75%, respectively. The cat cystatin allergen sequence contained a conserved cysteine protease inhibitor motif and two of three lipocalin domains. The sequence also had one potential N-linked glycosylation site.

Because dog allergens, Can f 1 and Can f 2, were known to be members of the lipocalin protein family, we compared the amino acid sequence of cat cystatin with the Can f 1 and Can f 2 sequences. The cat cysteine protease inhibitor motif was also partially conserved in the dog allergen sequences. Recombinant cat cystatin was obtained from bacterial lysates by affinity purification. This

purified protein migrated as a single band of ~ 11 kD on an 8–24 gradient SDS-PAGE. Molecular modeling showed that cat cystatin consists mainly of two and a half turn  $\alpha$ -helix, a five-stranded antiparallel  $\beta$ -pleated sheet interconnected by a hairpin loop and an additional carboxy terminal strand.

## Discussion

Cystatins are cysteine protease inhibitors whose main physiological function is believed to be to protect the host from the harmful effects of free cysteine proteases by rapidly trapping these enzymes in tight complexes. The cystatin superfamily is divided into three subgroups; the stefins, the cystatins and the kininogens. Stefins (also called family I cystatins) are small, acidic proteins, consisting of about 100-amino acid residues and lacking disulfide bonds. On the other hand, the cystatins (also called family II cystatins) are somewhat larger proteins, consisting of about 115-amino acid residues and having two disulfide bridges. Cat cystatin lacks cysteine residues and is thought to belong to the stefin subgroup.

Most animal allergens belong to the lipocalin family of ligand binding proteins or are albumins. We have identified a cysteine protease inhibitor allergen, cystatin, that is produced by cats. Allergens with similar functions are also produced by dogs [7]. It has been reported that the cysteine protease activity of mite allergen Der p 1 makes a significant contribution to its allergenicity [8, 9]. Our results raise the possibility that the high levels of cat (or dog) cysteine protease inhibitor allergens found in houses may serve to inactivate any potential protease activity of Der p 1 in the home environment.

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