

Cross-reactivity of *Anisakis simplex*: possible role of Ani s 2 and Ani s 3

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Abstract

Background *Anisakis simplex* is a fish parasite that can cause allergy in humans. The multiple cross-reactivities of this nematode make diagnostic tests for allergy unreliable, because of frequent false-positive results. To date, only four allergens of *A. simplex* have been characterized, but their role in cross-reactivity is largely unknown.

Methods We performed an amino acid sequence homology analysis, according to the Food and Agriculture Organization/World Health Organization (FAO/WHO) criteria, to identify allergens likely to be cross-reactive with Ani s 2 (paramyosin) and Ani s 3 (tropomyosin) of *A. simplex*. Next, within segments shared by Ani s 2 or Ani s 3 and homologous proteins, we searched for the occurrence of binding motifs of human leukocyte antigen (HLA) alleles (DRB1*1502, DQB1*0601, or DRB1*0404) known to be genetic risk factors for the development of allergy to *A. simplex*.

Results We identified a number of proteins belonging to various organisms, which are significantly similar to (and therefore are likely to cross-react with) Ani s 2 and Ani s 3. The presence of binding motifs of HLA DRB1*0404 in Ani s 2, Ani s 3, and homolog proteins also supports our hypothesis.

Conclusions Our results provide a possible molecular explanation of some cross-reactivities reported for *A. simplex*, and suggest additional ones.

Introduction

Anisakis simplex is a parasite of marine organisms. Once known only as a cause of gastrointestinal parasitosis in humans, it has recently become a "hot topic" as a frequent cause of cutaneous, respiratory, and/or systemic allergic reactions in consumers of raw/undercooked fish and in subjects professionally exposed to seafood.¹ The 96 papers published as of 18 June 2005 show the great scientific interest in *Anisakis*-related allergy, including improvement of diagnostic techniques. The diagnosis of allergy to *A. simplex* remains difficult, however, particularly because of the high prevalence of false-positive results, mainly due to the cross-reactivities of *A. simplex*.² Current laboratory techniques do not allow extensive searches for possible cross-reactive proteins and many allergens are not well characterized.

We aimed to better define the role played in cross-reactivity by two fully characterized allergens of *A. simplex*, paramyosin (Ani s 2) and tropomyosin (Ani s 3), using amino acid sequence analysis according to the Food and Agriculture Organization/World Health Organization (FAO/WHO) recommendations.

Materials and Methods

We extracted the amino acid sequence of Ani s 2 and Ani s 3 from the Entrez Protein database, and performed sequence comparison with all allergens of the same database ($n = 5598$ as of 18 June 2005) using protein-protein BLAST (Basic Local Alignment Search Tool).³ Selection of allergens in the database was made with the query "allergen OR IgE" followed by manual revision of the results. BLAST performs a residue-by-residue comparison between two proteins, giving each couple of residues a homology score based on the degree of chemical similarity. This score is used to calculate the E parameter, which describes the number of hits that can be "expected" to be found by chance when searching a database of a particular size. Although $E < 1$ is significant, we elected to be more stringent. Accordingly, only homologies with $E < 0.001$ were considered and, for each, the percentage identity (matching amino acids) and similarity (identical plus chemically similar amino acids) were recorded.

The FAO/WHO parameters for the *in silico* identification of potential new allergens⁴ were then used to define the potential allergic cross-reactivity of the homologous proteins found. According to these rules, proteins with more than 35% identity in a shared segment of 80 amino acids, or sharing a sequence of at least six consecutive amino acids, are very likely to be cross-reactive.

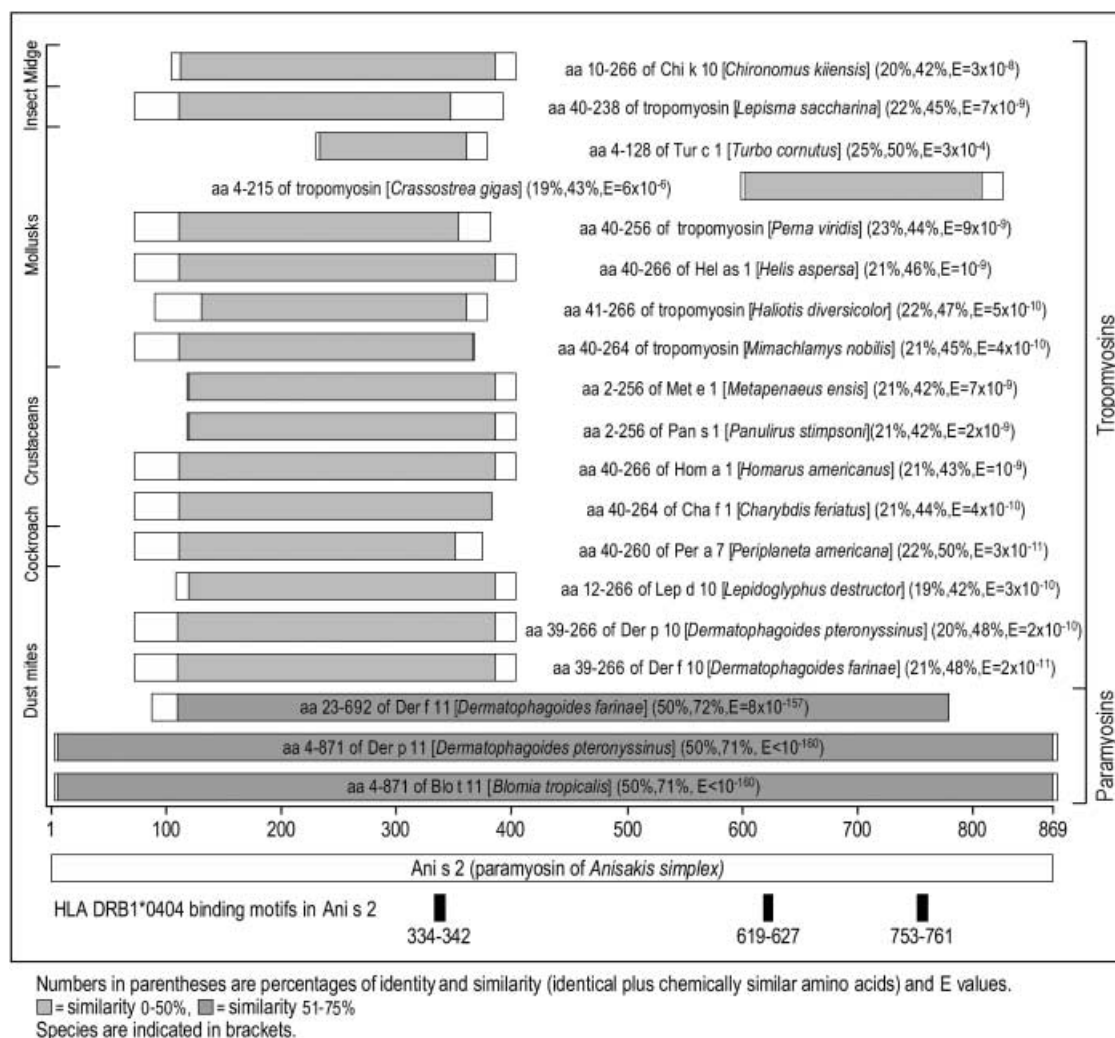


Figure 1 Extension and localization of significant homologies between *Anisakis 2* and other allergens. Homologous segments are marked in gray (light gray, similarity 0–50%; medium gray, similarity 51–75%) and nonhomologous segments in white. Occurrences of the binding motif of major histocompatibility complex (MHC) molecules coded by human leukocyte antigen (HLA) DRB1*0404 are also shown

Finally, the amino acid sequences of *Anisakis 2*, *Anisakis 3*, and homolog proteins were analyzed for their content of known T-cell-binding motifs of major histocompatibility complex (MHC) molecules coded by human leukocyte antigens (HLAs) DRB1*1502 (KPFQLEAVFEANQNT), DQB1*0601 (AKKQVEKDLANLTAE LDKVKE), and DRB1*0404 (V/I/L/M,X,X,F/Y/W/I/L/V/M/A/D/E,X,N/T/S/Q/R,X,X,K). Based on the sole paper available on the topic,⁵ these alleles are significantly over-represented (and hence are genetic risk factors) in subjects allergic to *A. simplex* relative to the general population.

Results

Both *Anisakis 2* and *Anisakis 3* showed significant homology with 19 known allergens (three paramyosins and 16 tropomyosins).

As expected, *Anisakis 2* (paramyosin of *A. simplex*) showed the highest levels of homology with paramyosins (identity, 50% always; similarity, 71–72%; maximum $E = 8 \times 10^{-157}$), and *Anisakis 3* (tropomyosin of *A. simplex*) showed the highest levels of homology with tropomyosins (identity, 46–75%; similarity, 61–89%; E between 4×10^{-94} and 3×10^{-25}). All homologies met one or both FAO/WHO criteria for significant allergen similarity ($> 35\%$ identity on an 80-amino-acid segment or six consecutive identical amino acids).⁴ Homologies of *Anisakis 2* with tropomyosins and of *Anisakis 3* with paramyosins were of lower magnitude (identity, 19–23% and 21–22%; similarity, 42–50% and 41–43%; E between 2×10^{-11} and 3×10^{-4} , and 10^{-8} and 6×10^{-8} , respectively). Figures 1 and 2 show the extension and localization of the segments shared by the above proteins with the two allergens *Anisakis 2* and *Anisakis 3*, respectively.

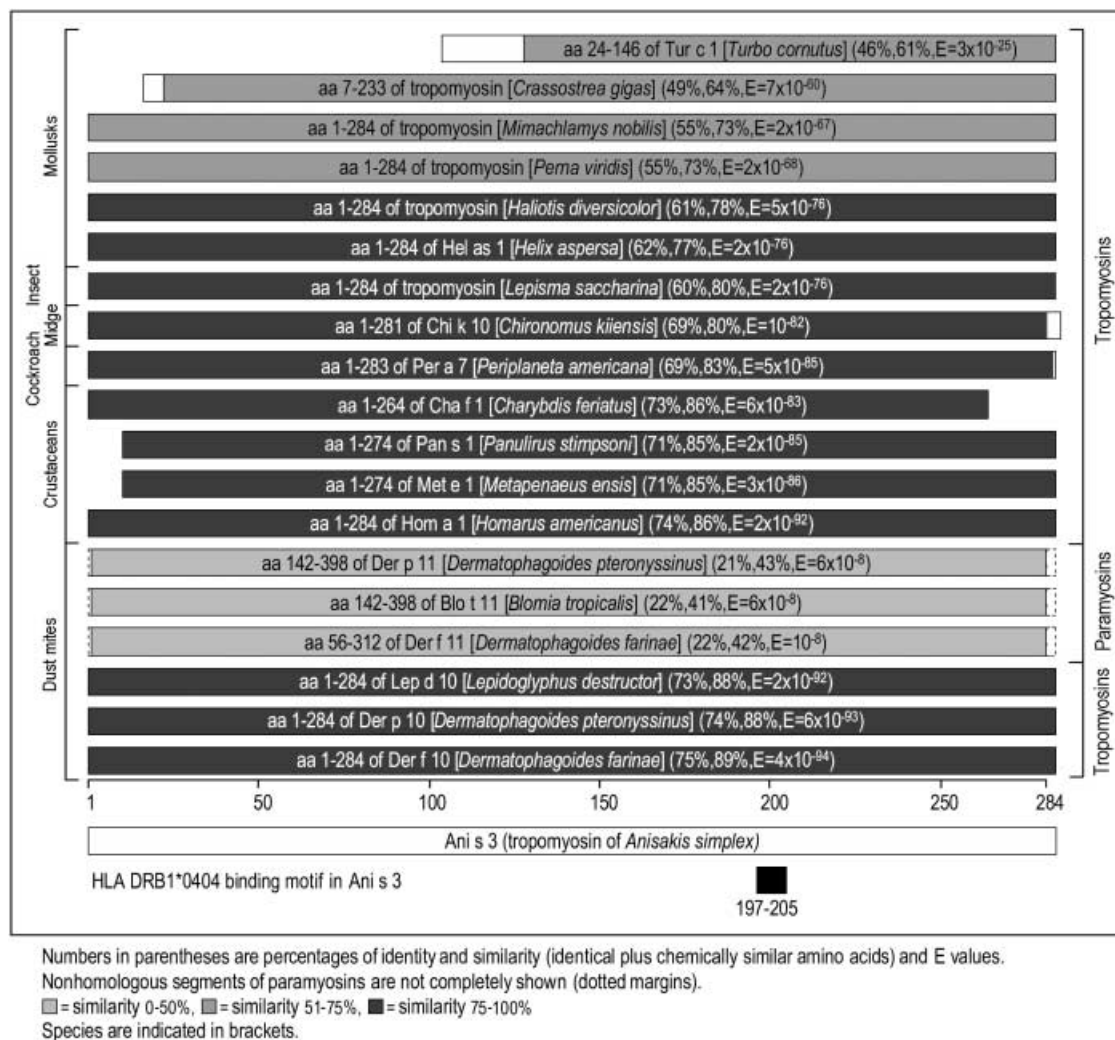


Figure 2 Extension and localization of significant homologies between Ani s 3 and other allergens. Homologous segments are marked in gray (light gray, similarity 0–50%; medium gray, similarity 51–75%; dark gray, similarity 76–100%) and nonhomologous segments in white. Occurrences of the binding motif of major histocompatibility complex (MHC) molecules coded by human leukocyte antigen (HLA) DRB1*0404 are also shown

The epitope-binding motifs of MHC molecules coded by HLAs DRB1*1502 and DQB1*0601 were not found in Ani s 2, Ani s 3, or the 19 homologous allergens. In contrast, the motif “V/I/L/M,X,X,F/Y/W/I/L/V/M/A/D/E,X,N/T/S/Q/R,X,X,K” (corresponding to HLA DRB1*0404) was present at least once in the homologous segments of all the aforementioned proteins of *A. simplex* and other organisms, except for tropomyosin of *Mimachlamys nobilis* (Table 1).

Discussion

Several studies have reported cross-reactivity between *A. simplex* and other organisms, such as parasites, dust mites, *Blatella germanica* (cockroach), *Clonorchis sinensis* (Chinese

or oriental liver fluke), *Daphnia* (commonly known as “water flea”), *Chironomid* spp. (commonly known as blood worm or midge), and *Pandalus borealis* (Northern or pink shrimp).^{6–9} Cross-reacting allergens have not been identified; at best, only their molecular weight has been defined. Our data identify some allergens likely to be responsible for part of the above cross-reactions, and allow us to hypothesize about possible new cross-reactive allergens.

The extensive and highly significant similarities of Ani s 2 agree with the experimental evidence of cross-reactions between *A. simplex* and some dust mites,⁶ and predict possible cross-reactivity with *Blomia tropicalis*, another dust mite common in tropical and subtropical areas.

With regard to Ani s 3, our data partly explain the known cross-reactivity with dust mites,⁶ and suggest a large number

Table 1 Occurrence of the motif “V/I/L/M,X,X,F/Y/W/I/L/V/M/A/D/E,X,N/T/S/Q/R,X,X,K” [binding motif of major histocompatibility complex molecules coded by human leukocyte antigen (HLA) DRB1*0404] in Ani s 2, Ani s 3, and significantly ($E < 0.001$) homologous allergens

Allergen and organism	Occurrence of the HLA DRB1*0404 binding motif (V/I/L/M,X,X,F/Y/W/I/L/V/M/A/D/E,X,N/T/S/Q/R,X,X,K)	
	Number of copies	Position(s) in the allergen sequence
Ani s 2 (paramyosin) of <i>Anisakis simplex</i> [nematode]	3	334–342, 319–627, 753–761
Ani s 3 (tropomyosin) of <i>Anisakis simplex</i> [nematode]	1	197–205
Blo t 11 (paramyosin) of <i>Blomia tropicalis</i> [dust mite]	3	156–164, 279–287, 701–709
Der p 11 (paramyosin/HDM allergen) of <i>Dermatophagoides pteronyssinus</i> [dust mite]	4	156–164, 167–175, 279–287, 701–709
Der f 11 (paramyosin) of <i>Dermatophagoides farinae</i> [dust mite]	4	70–78, 81–89, 193–201, 615–623
Der f 10 (tropomyosin) of <i>Dermatophagoides farinae</i> [dust mite]	2	156–164, 212–220
Per a 7 (tropomyosin) of <i>Periplaneta americana</i> [cockroach]	3	141–149, 197–205, 225–233
Der p 10 (tropomyosin) of <i>Dermatophagoides pteronyssinus</i> [dust mite]	3	141–149, 197–205, 225–233
Lep d 10 (tropomyosin) of <i>Lepidoglyphus destructor</i> [dust mite]	3	141–149, 197–205, 225–233
Cha f 1 (tropomyosin) of <i>Charybdis feriatus</i> [crustacean]	3	141–149, 197–205, 225–233
Tropomyosin of <i>Mimachlamys nobilis</i> [mollusk]	0	–
Tropomyosin of <i>Haliotis diversicolor</i> [mollusk]	3	68–76, 197–205, 225–233
Hel as 1 (tropomyosin) of <i>Helix aspersa</i> [mollusk]	2	197–205, 225–233
Hom a 1 (tropomyosin) of <i>Homarus americanus</i> [crustacean]	3	141–149, 197–205, 225–233
Pan s 1 (tropomyosin) of <i>Panulirus stimpsoni</i> [crustacean]	3	131–139, 187–195, 215–223
Met e 1 (tropomyosin) of <i>Metapenaeus ensis</i> [crustacean]	3	131–139, 187–195, 215–223
Tropomyosin of <i>Lepisma saccharina</i> [insect]	5	39–47, 53–61, 141–149, 197–205, 218–226
Tropomyosin of <i>Perna viridis</i> [mollusk]	1	225–233
Chi k 10 (tropomyosin) of <i>Chironomus kiiensis</i> [midge]	3	141–149, 197–205, 225–233
Tropomyosin of <i>Crassostrea gigas</i> [mollusk]	2	146–154, 174–182
Tur c 1 (tropomyosin) of <i>Turbo cornutus</i> [mollusk]	1	98–106

of possible new cross-reactivities, mainly with crustaceans [*Homarus americanus* (American lobster), *Metapenaeus ensis* (greasyback shrimp), *Panulirus stimpsoni* (spiny lobster), and *Charybdis feriatus* (crucifix crab)], mollusks [*Helix aspersa* (European brown garden snail), *Haliotis diversicolor* (abalone), *Perna viridis* (green mussel), *Mimachlamys nobilis* (noble scallop), *Crassostrea gigas* (giant Pacific oyster), and *Turbo cornutus* (turban shell)], and midges (*Chironomus kiiensis*), but also with organisms such as *Periplaneta americana* (American cockroach) and *Lepisma saccharina* (silverfish). Except for Tur c 1 of *Turbo cornutus*, the significance levels for homologies are very high (from 4×10^{-94} to 7×10^{-60}), as is the span of the homology [corresponding to the whole length of Ani s 3 (284 residues) in nine proteins, and 92–99% of the length (262–283 residues) in the remaining three

proteins]. Although relatively shorter (157 amino acids), homology between Ani s 3 and Tur c 1 is highly significant ($E = 3 \times 10^{-25}$), so that the possible cross-reactivity between these proteins is also worthy of experimental investigation. Many tropomyosins of arthropods, that are very similar amongst themselves and to Ani s 3, have already been reported as cross-reacting,¹⁰ confirming the usefulness of our *in silico* approach.

The presence of the binding motif of HLA DRB1*0404 within Ani s 2 and Ani s 3 agrees with the high prevalence of this allele in subjects allergic to *A. simplex*.⁵ The presence of the same motif in nearly all the homologous allergens found, within segments similar to *A. simplex* allergens, further supports our inference that some cross-reactions may be due to the above Ani s 2 and/or Ani s 3 homologous proteins.

In silico analysis also provides useful hints to understand certain obscure aspects of allergy to *Anisakis*. According to some authors, allergens are expressed only by live third-stage larvae of *A. simplex*. On this basis, as larvae are thermosensible, the cooking of seafood at a minimum of 60 °C for 10 min is recommended to prevent allergic reactions.¹⁰ Other authors, however, have reported unambiguous cases of allergy after the ingestion of correctly cooked fish.^{11–14} The analysis of the properties of the Ani s 3 homologous allergens suggests a possible explanation: Cha f 1 is reported to be a “heat-stable allergen tropomyosin”, and the high level of similarity suggests that *A. simplex* tropomyosin could share this feature. Thus, subjects sensitized to Ani s 3 could experience allergic reactions after ingesting properly cooked fish that is parasitized by *Anisakis*, because heating would not denaturate this allergen. The low rate of sensitization to Ani s 3 was explained by Asturias *et al.*,¹⁵ who reported that “high-dose exposure of the allergen under the harsh conditions of the gastrointestinal tract is necessary for sensitization,” and this occurs only in particular circumstances.

Conclusions

Allergy to *A. simplex* still presents many obscure aspects. Multiple cross-reactivities make diagnosis insufficiently reliable, and prevention not always feasible. Only a few allergens have been fully characterized, and their relevance, cross-reactivities, and, consequently, potential diagnostic usefulness are almost unknown. We provide a possible explanation of some of the cross-reactivities reported for *A. simplex*, and suggest additional ones. Our data also allow us to propose a pathogenic mechanism for allergic reactions to dead larvae of the parasite.

Amino acid sequence homology, even if highly significant, does not always equate to cross-reactive immunoglobulin E binding, presumably because even minor amino acid substitutions can cause conformational changes in the three-dimensional epitopes recognized by antibodies: this is the case, for example, for the C-terminal sequences of the pollen allergens Cyn d 1 and Lol p 1.¹⁶ Thus, our findings and, in general, all data produced by *in silico* methods should be considered not as a substitute, but as a support and guide, for “traditional”, irreplaceable, clinical and laboratory research.

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