

Clinical monosensitivity to salmonid fish linked to specific IgE-epitopes on salmon and trout beta-parvalbumins

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Fish-allergic patients often react to multiple fish species (1). This high cross-reactivity has been explained by a predominant sensitization to conserved epitopes of parvalbumin, the major fish allergen (2). A few cases of monosensitivity to single fish species have been reported (3–5). In these studies, the responsible allergens differed from parvalbumins.

We report the case of a 21-year-old female patient who experienced recurring episodes of fish allergy over many years. Swelling of the tongue and oral mucosa, edema of the face and vomiting occurred a few minutes after the ingestion of raw and cooked salmon or trout. The patient reported allergic reactions upon eating traces of these two salmonid fish. She experienced no reaction with other fish such as cod, pollock or tuna.

Skin prick tests (SPT) performed with commercial extracts (Stallergènes,

Antony, France) were strongly positive for salmon and trout, but negative for cod, sardine, and whiting. The total IgE level was 140 kU/l (Phadia ImmunoCAP System, Uppsala, Sweden). Specific IgE were positive for salmon (0.36 kU/l).

Proteins were extracted from fish muscle of salmon, trout, cod, carp, mackerel, redfish and herring. IgE immunoblotting with the patient's serum detected a distinct 10-kDa protein band only in salmon and trout extract (Fig. 1A). Seven native parvalbumins were purified by a 2-step chromatographic procedure. Brook and rainbow trout parvalbumins were cloned using rapid amplification of cDNA ends (RACE) PCR technology and submitted to databases (FM994927, FM994928, FN544258, FN544259). Brook and rainbow trout DNA sequences were different in few positions, but protein translation resulted in identical parvalbumins. Salmon parvalbumins were cloned using database sequences (X97824, X97825). Recombinant proteins were expressed in *Escherichia coli* M15. Protein comparison of salmon and trout parvalbumin isoforms showed high identities among beta-1 (96%) and beta-2 homologs (97%). IgE immunoblot analysis with purified native parvalbumins, recombinant parvalbumins (salmon, trout) and food-grade fish gelatin (Rousselot, Courbevoie, France) was performed using patient serum. Salmon and trout parvalbumins were strongly positive, but cod and carp parvalbumins were negative (Fig. 1A). Furthermore, IgE did not recognize mackerel, redfish and herring parvalbumin or fish gelatin. IgE binding to salmon parvalbumin was abolished by preincubation of the serum with trout parvalbumin, but not with cod or carp parvalbumin (data not shown). Specific IgE binding was quantified by enzyme-linked immunosorbent

assay (ELISA) using purified parvalbumins in the presence of 0.5 mM calcium chloride. IgE binding was similar for salmon parvalbumin and trout homologs (Fig. 1B). IgE titers decreased to <0.1 kU/l upon calcium depletion. No IgE reactivity was found for other parvalbumins or fish gelatin.

In conclusion, we could show that the IgE antibodies of a patient clinically mono-allergic to salmon and trout bound to parvalbumins of these two salmonid fishes, but not to parvalbumin of cod, carp, mackerel, redfish and herring. Comparison of salmon parvalbumin to the trout homolog showed that the beta-1 isoforms differed by four and the beta-2 isoforms by three amino acids only. When aligning the salmonid beta-1 parvalbumins to those of carp, cod and mackerel, the C-terminal regions at positions 3–10 and 27–38 were identical for salmonid parvalbumins, but different compared to the proteins of the other fishes. These two regions could possibly be part of the B-cell epitopes recognized specifically by the patient's IgE antibodies on salmon and trout parvalbumin. This report illustrates also the importance of using a panel of parvalbumins from different fish species to perform component-resolved diagnostic work-up of fish allergy. In clinical practice, this will help advising fish-allergic patients which fish species to consume, preferably after confirmation by *in-vivo* provocation tests.

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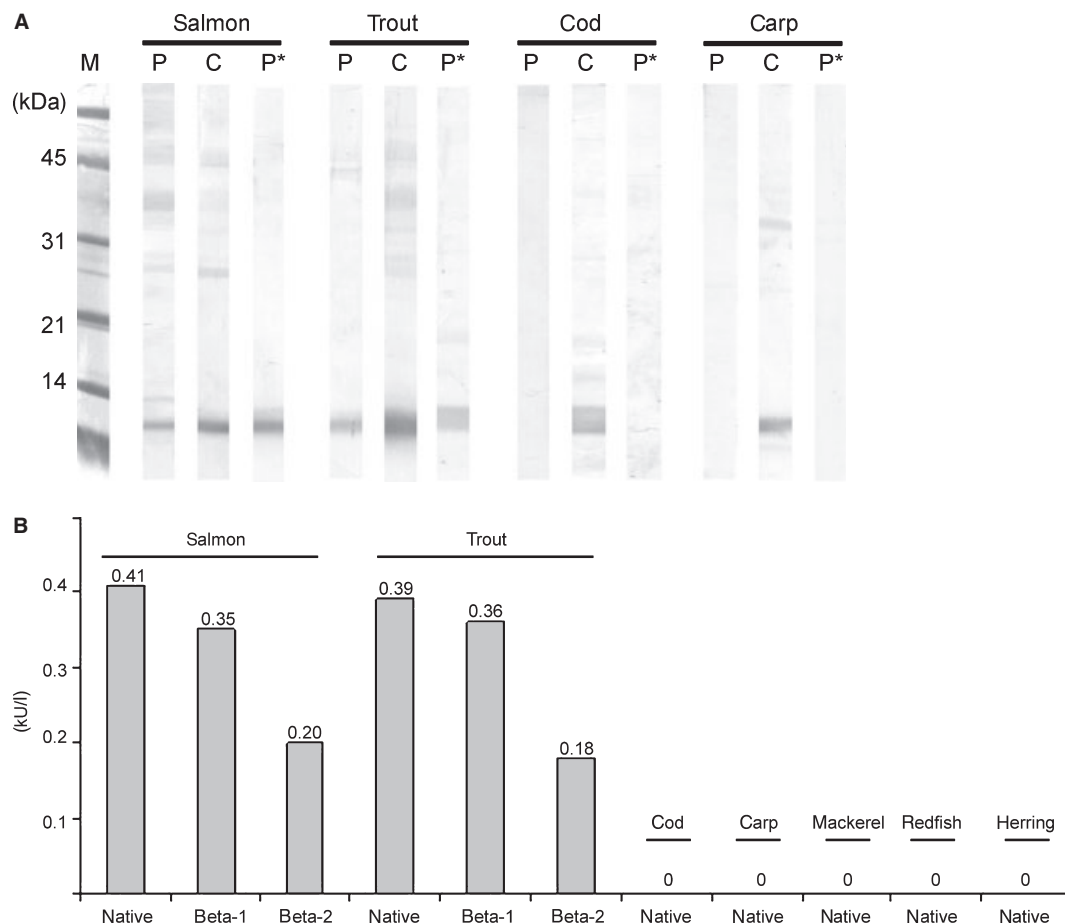


Figure 1 IgE analysis of salmon and trout parvalbumins. (A) IgE immunoblot using patient serum (P). A 10-kDa allergen was detected in the extract of salmon and trout. IgE bound specifically to purified parvalbumin (P*) from salmon and trout, but not from cod and carp. Positive controls (C) were performed using an anti-parvalbumin antibody (Swant, Bellinzona, Switzerland). (B) IgE quantification by ELISA. Similar titers were obtained for native parvalbumin from salmon and trout as well as for recombinant salmon and trout parvalbumin isoforms. No IgE was bound to native cod, carp, mackerel, redfish or herring parvalbumin.

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