characterization of select edible nut seed proteins. J Agric Food Chem 2009; 57(17):7846-56).

In the absence of specific allergens, it is not feasible to provide a sufficiently realiable diagnosis. Whole nut extracts or partially purified extracts are associated with limitations similar to the shortcomings of skin prick assays such as instability and insufficient specificity, more specifically false negative results.

Therefore, the problem underlying the present invention is to provide a method for the diagnosis of an allergy, preferably a nut allergy, more preferably a macadamia allergy, and reagents for such a method, which method has an improved diagnostic reliability, in particular sensitivity, when compared to state of the art assays.

Another problem underlying the present invention is to provide an assay for the differential diagnosis of allergies, preferably nut allergies such as tree nut allergies, more preferably for distinguishing an allergy to a macadamia nut from an allergy to a nut other than macadamia, and/or for monitoring the therapy of an individual suffering from such an allergy.

Another problem underlying the present invention is to provide reagents useful for such an assay, methods for making such reagents and methods for such diagnosis. Another problem underlying the present invention is to provide a method of treatment and a pharmaceutical composition for treating patients suffering from an allergy to a macadamia nut.

The problem underlying the present invention is solved by the subject-matter of the attached independent and dependent claims.

Summary

In one aspect, the present disclosure refers to a diagnostically useful carrier comprising a polypeptide comprising a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2 or a variant thereof having the ability to bind to a macadamia nut allergy-specific IgE antibody, wherein the variant has at least 80 % sequence identity with amino acid residues 21-645 of SEQ ID NO2, wherein the variant may in addition comprise a covalent chemical modification selected from the group comprising glycosylation, phosphorylation, acetylation, decarboxylation, citrullination and hydroxylation, wherein the polypeptide is a purified, isolated or recombinant polypeptide, wherein the diagnostically useful carrier is selected from the group comprising a bead, a test strip, a microtiter plate, a

blot, preferably from the group comprising western blot, line blot and dot blot, a glass surface, a slide, a biochip, a membrane, a microarray, an electrophoresis gel and a microtiter plate.

In another aspect, the present disclosure refers to an *in vitro* method for the diagnosis of a macadamia nut allergy comprising the step detecting in a sample obtained, preferably from a mammal, more preferably a human, an antibody binding to the macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2.

In yet another aspect, the present disclosure refers to a test kit for the diagnosis of a disease, comprising the diagnostically useful carrier as described herein, one or more solutions from the group comprising sample dilution buffer, washing buffer and a buffer comprising a means for specifically detecting any specifically detected antibody, preferably a secondary antibody and optionally a means for detecting a complex comprising the polypeptide and an antibody binding to a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2.

In yet another aspect, the present disclosure refers to a polypeptide comprising a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2 or a variant thereof having the ability to bind to a macadamia nut allergy-specific IgE antibody, wherein the variant has at least 80 % sequence identity with amino acid residues 21-645 of SEQ ID NO2, wherein the variant may in addition comprise a covalent chemical modification selected from the group comprising glycosylation, phosphorylation, acetylation, decarboxylation, citrullination and hydroxylation, wherein the polypeptide is a purified isolated or recombinant polypeptide, for use in therapy in the diagnosis of a macadamia nut allergy.

Detailed Description

In a first aspect, the problem underlying the present invention is solved by a polypeptide comprising a macadamia protein selected from the group comprising a protein running in a 2D SDS PAGE at pH 6.3 to 8.7 and 53 to 67 kDa and a protein running in a 2D SDS PAGE at pH 6.5 to 7.9 and 20 to 25 kDa or a variant thereof, preferably the protein running at pH 6.3 to 8.7 and 53 to 67 kDa or a variant thereof. The polypeptide is a polypeptide from *Macadamia tetraphylla* or *M. integrifolia, preferably M. integrifolia.*

In a preferred embodiment, the polypeptide is an isolated and/or recombinant polypeptide.

In another preferred embodiment, the polypeptide is a fusion polypeptide, preferably comprising a tag for detection or purification.

In a second aspect, the problem is solved by a nucleic acid encoding the polypeptide according to the present invention, preferably in a vector, more preferably in a vector in a cell.

In another preferred embodiment, the polypeptide is immobilized.

In a 3rd aspect, the problem is solved by a medical device comprising the polypeptide according to the present invention, wherein the medical device is preferably a diagnostically useful carrier, more preferably selected from the group comprising a bead, a test strip, a microtiter plate, a blot, preferably from the group comprising western blot, line blot and dot blot, a glass surface, a slide, a biochip, a membrane, a microarray, an electrophoresis gel and a microtiter plate.

In a 4th aspect, the problem is solved by a method comprising the step detecting in a sample, preferably from a mammal, more preferably a human, an antibody binding to the polypeptide according to the present invention, which method is preferably for the diagnosis of a nut allergy, more preferably for the diagnosis of a macadamia allergy.

In a 5th aspect, the problem is solved by a method for the treatment of a disease comprising the step administrating to a subject or contacting with a sample from a subject the polypeptide according to the present invention,

wherein the disease is preferably a nut allergy, more preferably a tree nut allergy, most preferably a macadamia allergy.

In a 6th aspect, the problem is solved by an antibody binding to a macadamia protein selected from the group comprising a protein running in a 2D SDS PAGE at pH 6.3 to 8.7 and 53 to 67 kDa and a protein running in a 2D SDS PAGE at pH 6.5 to 7.9 and 20 to 25 kDa,

preferably the protein running at pH 6.3 to 8.7 and 53 to 67 kDa,

Claims

1. A diagnostically useful carrier comprising a polypeptide comprising a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2

   or a variant thereof having the ability to bind to a macadamia nut allergy-specific IgE antibody, wherein the variant has at least 80 % sequence identity with amino acid residues 21-645 of SEQ ID NO2, wherein the variant may in addition comprise a covalent chemical modification selected from the group comprising glycosylation, phosphorylation, acetylation, decarboxylation, citrullination and hydroxylation,

   wherein the polypeptide is a purified, isolated or recombinant polypeptide,

   wherein the diagnostically useful carrier is selected from the group comprising a bead, a test strip, a microtiter plate, a blot, preferably from the group comprising western blot, line blot and dot blot, a glass surface, a slide, a biochip, a membrane, a microarray, an electrophoresis gel and a microtiter plate.

2. An *in vitro* method for the diagnosis of a macadamia nut allergy comprising the step detecting in a sample obtained, preferably from a mammal, more preferably a human, an antibody binding to the macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2.

3. A test kit for the diagnosis of a disease, comprising the diagnostically useful carrier according to claim 1, one or more solutions from the group comprising sample dilution buffer, washing buffer and a buffer comprising a means for specifically detecting any specifically detected antibody, preferably a secondary antibody and optionally a means for detecting a complex comprising the polypeptide and an antibody binding to a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2.

4. The method according to claim 2, wherein the sample is a bodily fluid comprising antibodies, preferably selected from the group comprising whole-blood, serum, cerebrospinal fluid and saliva, more preferably whole blood.

5. The method according to any one of claims 2 or 4 and the test kit according to claim 3, wherein the antibody is an IgE antibody.

6. A polypeptide comprising a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2

   or a variant thereof having the ability to bind to a macadamia nut allergy-specific IgE antibody, wherein the variant has at least 80 % sequence identity with amino acid residues 21-645 of SEQ ID NO2, wherein the variant may in addition comprise a covalent chemical modification selected from the group comprising glycosylation, phosphorylation, acetylation, decarboxylation, citrullination and hydroxylation,

   wherein the polypeptide is a purified isolated or recombinant polypeptide,

   for use in therapy in the diagnosis of a macadamia nut allergy.

7. The diagnostically useful carrier, method, kit or polypeptide according to any of claims 1 to 6, wherein the polypeptide is a fusion polypeptide, preferably comprising a tag for detection or purification.

8. The diagnostically useful carrier, method, kit or polypeptide according to any of claims 1 to 7, wherein the polypeptide is immobilized.