(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 526 422 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
25.05.2016 Bulletin 2016/21

(21) Application number: **11735215.3**

(22) Date of filing: **21.01.2011**

(51) Int Cl.:
G01N 33/68 *(2006.01)* ***G01N 33/564*** *(2006.01)*

(86) International application number:
PCT/US2011/021992

(87) International publication number:
WO 2011/091220 (28.07.2011 Gazette 2011/30)

(54) **METHOD OF DIAGNOSING SJOGREN'S DISEASE**

VERFAHREN ZUR DIAGNOSE DES SJÖGREN-SYNDROMS

PROCÉDÉ DE DIAGNOSTIC DE LA MALADIE DE SJÖGREN

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **21.01.2010 US 297167 P**

(43) Date of publication of application:
28.11.2012 Bulletin 2012/48

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- **K BAYETTO AND R.M. LOGAN: "Sjögren's syndrome: a review of aetiology, pathogenesis, diagnosis and management", AUSTRALIAN DENTAL JOURNAL, vol. 55, 1 June 2010 (2010-06-01), pages 39-47, XP055062570, Australian Dental Asociation, St Leonards NSW Australia ISSN: 0045-0421, DOI: 10.1111/j.1834-7819.2010.01197.x**
- **RUAN ET AL.: 'The Autoimmune Regulator Directly Controls the Expression of Genes Critical for Thymic Epithelial Function' J. IMMUNOL. vol. 178, 2007, pages 7173 - 7180, XP008164600**
- **HU ET AL.: 'Salivary Proteomic and Genomic Biomarkers for Primary Sjogren?s Syndrome' ARTHRITIS & RHEUMATISM vol. 56, no. 11, November 2007, pages 3588 - 3600, XP008164621**

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Description

BACKGROUND OF THE INVENTION

[0001] Sjogren's disease is a common autoimmune disorder with significant morbidity and mortality secondary to destruction of the salivary and lachrymal glands. It involves both local and systemic autoimmunity and is generally recognized only after salivary and lachrymal glands are destroyed resulting in dry mouth and dry eyes (Borchers, A. T., S. M. Naguwa, C. L. Keen, and M. E. Gershwin. 2003. Immunopathogenesis of Sjogren's syndrome. Clin Rev Allergy Immunol 25:89-104; Delaleu, N., R. Jonsson, and M. M. Koller. 2005. Sjogren's syndrome. Eur J Oral Sci 113:101-113). Sjögren's syndrome is a common syndrome affecting 0.5% of the population with a strong female predominance (Delaleu, N., R. Jonsson, and M. M. Koller. 2005. Sjogren's syndrome. Eur J Oral Sci 113:101-113, Fox, R. I. 2005. Sjogren's syndrome. Lancet 366:321-331.). It consists of xerostomia and xerophthalmia and may be due to several causes including aging, infections, medications, environmental toxins and autoimmune responses (Daniels, T. E. 2000. Evaluation, differential diagnosis, and treatment of xerostomia. J Rheumato/ Suppl 61:6-10). Sjögren's disease is a primary disorder consisting of Sjogren's syndrome with systemic manifestations including lymphadenopathy, interstitial pneumonitis and mild renal disease (Borchers, A. T., S. M. Naguwa, C. L. Keen, and M. E. Gershwin. 2003. Immunopathogenesis of Sjogren's syndrome. Clin Rev Allergy Immunol 25:89-104; Delaleu, N., R. Jonsson, and M. M. Koller. 2005. Sjogren's syndrome. Eur J Oral Sci 113:101-113). Sjögren's syndrome is often seen in association with other autoimmune diseases, especially systemic lupus erythematosus (SLE) (Manoussakis, M. N., et al. Moutsopoulos. 2004. Sjogren's syndrome associated with systemic lupus erythematosus: clinical and laboratory profiles and comparison with primary Sjogren's syndrome. Arthritis Rheum 50:882-891). Patients with Sjögren's disease often have hypergammaglobulinemia, and various autoantibodies, especially to Ro and La (Fox, R. I. 2005. Sjogren's syndrome. Lancet 366:321-331, Lazarus, M. N., and D. A. Isenberg. 2005. Development of additional autoimmune diseases in a population of patients with primary Sjogren's syndrome. Ann Rheum Dis 64:1062-1064, ansen, A., P. E. Lipsky, and T. Dorner. 2005. Immunopathogenesis of primary Sjogren's syndrome: implications for disease management and therapy. Curr Opin Rheumatol 17:558-565). Almost 4% of patients with Sjögren's disease will develop lymphoma, predominantly B cell non-Hodgkin lymphomas.

[0002] The diagnosis of Sjogren's disease is generally made when dry eyes causes irritation and corneal abrasions, and dry mouth causes difficulty swallowing and dental caries.

Biochemical diagnosis is based on detection of lymphocytes infiltrating the salivary glands and serum auto antibodies directed towards Ro and La. Current therapies involve the use of artificial tears and saliva as well as cholinergic drugs to enhance secretions from the few remaining glandular cells (the disclosure of the following three citations are incorporated herein by reference: Kassan, S. S., and H. M. Moutsopoulos. 2004. Clinical manifestations and early diagnosis of Sjogren syndrome. Arch Intern Med 164:1275-1284, Latkany, R. 2008. Dry eyes: etiology and management. Current Opinion in Ophthalmology 19:287-291, Thanou-Stavraki, A., and J. A. James. 2008. Primary Sjogren's syndrome: Current and prospective therapies. Seminars in Arthritis and Rheumatism 37:273-292). However, no current therapies restore salivary and lachrymal gland function because the glands are largely destroyed by the time the disease is identified. It would therefore be of great benefit to be able to diagnose Sjogren's disease early since that is when it is amenable for treatment, but no such diagnostic methods exist. Thus, there is an ongoing and unmet need for improved methods for diagnosing Sjogren's disease, and in particular for use in diagnosis before the diseases progresses to a point where current therapeutic approaches are inadequate.

US 2007/0184502 relates to a method of diagnosing Sjögren's syndrome wherein the presence and/or the amount of IgA autoantibodies against α-fodrin is determined in a sample.

Ruan et. al. "The Autoimmune Regulator Directly Controls the Expression of Genes Critical for Thymic Epithelial Function" (J. Immunol., vol. 178, 2007, pages 7173-7180) teaches that SP-1 and at least eight other proteins are expressed to a lesser degree in mice which have a homozygous deletion of an autoimmune regulator gene (Aire) relative to mice which are homozygous normal for Aire.

US2007/184511 relates to a method for diagnosing a person having Sjögren's syndrome, the method involving analyzing samples of body fluid or tissue with mass spectrometry and comparing whether the patient's sample contains m/z values that are characteristic of a Sjögren's syndrome reference database.

Hu et. al. "Salivary Proteomic and Genomic Biomarkers for Primary Sjögren's Syndrome" (Arthritis & Rheumatism, vol. 56, no. 11, November 2007, pages 3588-3600) relates to the identification of protein and messenger RNA biomarkers in human whole saliva.

US 5895812 relates to novel polypeptides which provide secretion enhancing activity on lacrimal and parotid gland cells, monoclonal antibodies thereto, and methods of diagnosing Sjögren's syndrome using the antibodies.

K. Bayetto and R.M. Logan "Sjögren's syndrome: a review of aetiology, pathogenesis, diagnosis and management" (Australian dental journal, vol. 55, 1 June 2010, pages 39-47) provides a review of the manifestations associated with Sjögren's syndrome and the factors that have a role in its aetiology and pathogenesis.

SUMMARY OF THE INVENTION

[0003] The present invention provides a method for determining whether a human individual has Sjögren's disease (SD). The method comprises determining in a biological sample from the individual the presence of antibodies directed to salivary gland protein 1 (SP-1), parotid secretory protein (PSP) or, carbonic anhydrase 6 (CA6), or determining a combination of the antibodies. Determining that the individual has SD is based on the presence of the antibodies. Also provided is a method for determining that an individual does not have SD which comprises determining in a biological sample obtained from the individual the absence of detection of antibodies to PSP, and SP-1 and determining based on the absence of detection of the antibodies that the individual does not have SD. The individual may also have less antibodies to CA6 relative to an SD patient. Any PSP or CA6 protein may be used. However, there is no known human homologue to Sup-1, and the invention accordingly provides a novel and unexpected discovery that humans with SD produce autoantibodies that recognize non-human SP-1, and in particular murine SP-1. It is expected that SP-1 produced in other non-human mammals can also be used for detecting anti-SP-1 antibodies.

[0004] The method of the invention can be used to diagnose SD in any individual of any age or gender, and at any stage of the disease. In one embodiment, the invention is used to detect early SD.

[0005] The antibodies that are positively associated with SD and described further herein can be detected using any suitable method and/or reagents for detecting antibodies. In one embodiment, the antibodies are detected using an ELISA assay.

[0006] The invention also provides use of kits in the method of the invention comprising the antigens to which the antibodies of SD patients are directed and may further comprise components for biological sample collection, reagents for antibody detection, control reaction, and other materials useful for detecting antibodies. In one embodiment, the invention provides use of a kit comprising purified SP-1, PSP and CA6 proteins or fragments thereof that are recognized by antibodies produced by SD patients. The proteins may be provided in isolated and purified form, and they may be covalently or non-covalently associated with a solid matrix.

BRIEF DESCRIPTION OF THE FIGURES

[0007]

Figure 1 provides a photographic representation of Western blotting for autoantibodies in the sera of IL-14aTG mice during early stages of Sjogren's Disease. To obtain the data summarized in Figure 1, sera were collected from IL-14αTG mice, NOD and C57BL/6 mice at 7 months of age. Custom expressed and purified salivary gland protein 1 (SP-1), parotid secretory protein (PSP) and carbonic anhydrase 6 (CA6) were used to run Western blots with these sera. Data shown are representative of 6 mice studied in each group. The left panel shows the serum of an IL-14αTG mouse that recognizes CA6 and PSP strongly and SP-1 weakly. The middle panel shows the same study with the serum of a C57BL/6 mouse. C57BL/6 sera bound none of these autoantigens. The right panel shows the same study with the serum of an NOD mouse. Both CA6 and PSP are strongly recognized by this serum.

Figure 2 provides a photographic representation of Western blotting results demonstrating that lymphotoxin is found in the salivary gland secretions of IL-14a TG mice but not littermate controls.

Figure 3 provides a graphical representation of data showing that lymphotoxin is found in the sera of selected patients with Sjogren's disease.

Figure 4 provides a photographic representation of Western blotting showing autoantibodies in the sera and salivary glands of IL-14aTG.LTA-/- mice. The data show that the serum of an IL-14aTG.LTA-/- mouse at 11 months of age reacts with CA6 and PSP.

Figure 5 provides a photographic representation of Western blotting results showing that sera from Patients with Sjogren's Disease Contain Autoantibodies to CA6, PSP and SP-1. The Western blots were performed as in Figure 1 except sera were used from patients with Sjogren's disease or aged matched normal controls. Five patients and two normal controls were evaluated. Data shown are from one representative patient (all five patients showed similar results) and one normal control (both normal controls showed similar results). Patients with SD but not normal controls have antibodies to PSP and SP-1.

DESCRIPTION OF THE INVENTION

[0008] The present invention provides a method for determining whether a human individual has Sjögren's disease (SD). The method comprises determining in a biological sample obtained from the individual the presence of antibodies to salivary gland protein 1 (SP-1), parotid secretory protein (PSP), carbonic anhydrase 6 (CA6), or a combination of the SP-1, PSP and CA6 antibodies. The individual is identified as having SD if such antibodies or combinations thereof are present.

[0009] The invention provides the first identification of a positive correlation between SD and antibodies to any of SP-1, PSP and CA6. Our discovery that human SD patients produce antibodies that recognize murine SP-1 was particularly unexpected because there is no known human homologue to this protein. Thus, the invention provides a surprising and novel method for identifying individuals who have SD. The invention further provides for identifying an individual as not having SD if antibodies to SP-1 and PSP are not detected in a biological sample obtained from the individual.

[0010] In addition to antibodies to SP-1, PSP and CA6, antibodies to other markers can be determined in the method of the invention for evaluating whether or not an individual has SD. Non-limiting examples of such antibodies include those directed to lymphotoxin (LTA), mucin 10 (submandibular gland salivary mucin), salivary amylase 1, Ro, La, muscarinic receptor 3, fodrin, and the cytokines IL-14 and interferon-α.

[0011] Our demonstration that production of antibodies to SP-1, PSP and CA6 are indicative of SD is supplemented by research we performed on clinically relevant animal models of SD. In this regard, we have developed an animal model that reproduces all the immunological and clinical features seen in patients with SD in the same relative time frame. The animal model we developed is referred to as the IL-14alpha transgenic mouse (IL14aTG). Using this model we have demonstrated that lymphocytic infiltration of the salivary glands occurs after the glands have already been destroyed and that only a small percentage of the mice develop antibodies to Ro and La. Our data also demonstrate that LTA is important to early salivary gland injury in IL14aTG and the NOD mouse models of SD, and that IL-14aTG mice lacking LTA (IL-14aTG.LTA-/-) retain normal salivary gland function and suffer no lymphocytic infiltration of their salivary glands. We also demonstrate that LTA is found in the serum of human SD patients.

[0012] IL-14aTG mice, like patients with SD, produce interferon-α (IFN-a) systemically and lymphotoxin (LTA) locally in the salivary glands. Autoantibodies are deposited in the salivary gland at the time that salivary gland function is lost. The auto-antigens recognized at this stage are different than the auto-antigens seen later in the disease, Ro and La, which traditionally have been felt to be characteristic of SD. We have also demonstrated in this model that salivary gland function is lost before infiltration of the salivary glands with lymphocytes. In summary, and without intending to be bound by any particular theory, it is considered that IL-14aTG mice reproduce all the features of SD seen in patients in the same relative time frame. Further, SD occurs in all IL-14aTG mice tested. The time course of SD in IL-14aTG mice is 1) hypergammaglobulinemia and early antibody production at 6 months of age, 2) decreased salivary gland function with lymphocytic infiltration of only the submandibular glands, but antibody deposition in the submandibular and parotid glands at 10 months, 3) lymphocytic infiltration of the submandibular, parotid and lachrymal glands with B and T cells and plasma cells along with mild renal and lung disease at 14 months, and 4) large B cell lymphoma at 18 months. Note that loss of salivary gland function occurs several months before lymphocytic infiltration of the salivary glands, which indicates there is antibody and/or cytokine mediated injury that occurs before lymphocytic infiltration of the glands. Furthermore, as noted above, the IL-14aTG mice generally do not produce anti-Ro and anti-La antibodies during the early stages of the disease. The pattern of immunofluorescence for immunoglobulin deposition in the salivary glands varies over time, suggesting that different auto antigens are likely to be important at various stages of the disease. Additionally, IL-14aTG mice do not develop diabetes, like NOD mice, or proliferative glomerulonephritis, like (NZB x NZW) F1 and MRL/lpr mice. The IL-14aTG mouse is thus the only animal model of SD that develops all the features of Sjogren's disease in the absence of other autoimmune diseases. Accordingly, the IL-14aTG and IL-14aTG.LTA mice are valuable for identifying early events in the development of SD.

[0013] We identified the SD antigens disclosed herein in part by examining the expression of mRNA in the spleens ofIL14aTG mice. We have produced purified antigens encoded by these mRNAs and have shown that IL14aTG and NOD mouse models of SD develop antibodies to these antigens during the early course of their disease (Figure 1). We have also demonstrated that lymphotoxin is present in the salivary gland secretions ofIL14aTG mice during the early course of their disease, and that elimination of lymphotoxin prevents the development of salivary gland injury (Figure 2). We have shown that lymphotoxin is present in the sera of human patients with SD (Figure 3). Further, we have demonstrated the presence of antibodies to SD antigens in IL-14aTG.LTA-/- mice (Figure 4.) Further still, we also demonstrate that sera from human patients with SD contain autoantibodies to CA6, PSP and SP-1 (the latter of which as described above has no known human homologue) while normal controls do not have antibodies to PSP-1 or SP-1, and have only weak antibody response to CA6 (Figure 5). The antibody response to CA6 was stronger in SD patients than in normal controls.

[0014] Each of the SD markers to which antibodies are determined according to the method of the invention are well characterized and are known in the art and their coding and amino acid sequences are available in GenBank. Each GenBank reference presented in this disclosure is incorporated herein by reference as of the date of this invention. It is expected that any mammalian CA6, PSP and SP-1 antigens (with the caveat that there is no know human homologue to SP-1) can be used with any of a wide variety of established immunoassays in the method of the invention to determine whether or not an individual produces antibodies directed to the antigens. In one embodiment, murine PSP is described by the sequences presented in GenBank entry NM_008953.2. In one embodiment, murine SP-1 described by the sequences presented in GenBank entry NM_009267.2. In one embodiment, CA6 is described by the sequences presented in GenBank entry NM_009802.2.

[0015] The protein antigens that are used for detecting autoantibodies according to the method of the invention can be made using techniques well known to those skilled in the art. For example, any DNA sequence encoding the antigens can be made using standard techniques and inserted into any number of expression vectors. Suitable expression vectors have been described in the literature

and many are commercially available. Likewise, a wide variety of expression systems are known in the art and are commercially available, including prokaryotic and eukaryotic systems. The proteins can be isolated from the expression systems or any other suitable source and purified for use in the invention to any desired degree of purity.

[0016] The biological sample obtained from the individual can be any biological sample that comprises antibodies, and can comprise biological tissue and/or biological liquid. In various embodiments, the biological liquid is blood, serum or saliva.

[0017] The autoantibodies that are positively correlated with SD as described herein can be detected using any suitable technique, device, system and/or reagents, many of which are commercially available and/or are otherwise well know to those skilled in the art. In various, embodiments, suitable detection techniques include but are not necessarily limited to immunohistological techniques, Western blotting, multi-well assay plates adapted for detection of the antibodies, beads adapted for detection of the antibodies, a lateral flow device or strip that is adapted for detection of the antibodies, ELISA assays, or any modification of an ELISA assay that is suitable for detecting the antibodies. Further, any and alls isotypes of the antibodies can be detected. It is considered that the early antibodies are all or predominantly IgM and later antibodies are comprised of IgM and IgG. Thus, if desired, the invention can be adapted to discriminate between isotypes to assist in determining, for example, the stage of disease

[0018] The method of the invention is suitable for performing on a biological sample obtained from an individual of any age or gender. The method may be performed once, or a series of tests may be performed to, for example, monitor an individual's response to a treatment.

[0019] In one embodiment, the invention is suitable for determining early SD. Early SD is considered to be a stage of SD before salivary and/or lachrymal gland function is diminished to a point where clinical symptoms of SD become manifest. Those skilled in the art will be able to recognize early SD, particularly when provided the benefit of the present disclosure. Further, the invention provides in some individuals, such as those with other autoimmune diseases or a family history SD, testing for SD before any symptoms appear.

[0020] In one embodiment, the invention comprises fixing the result of performing the method of the invention in a tangible medium of expression, such as a digitized computer record. The invention further comprises communication the result of the performing the method of the invention to a health care provider.

[0021] The invention also provides use of kits in the method of the invention comprising the antigens to which the antibodies of SD patients are directed and may further comprise components for biological sample collection and reagents for antibody detection, positive controls, and the like. In one embodiment, the invention provides use of a kit comprising purified SP-1, PSP and CA6 proteins or fragments thereof that are recognized by antibodies produced by SD patients. Fragments of these proteins can be recognized by antibodies produced by individuals who have SD can be determined using routine skill if given the benefit of the present disclosure. In general, the fragments will be from 9 amino acids in length, up to one amino acid less than the full length the proteins, and including all integers from 9 amino acids up to one amino acid less than the full length of the proteins, inclusive. Each and every fragment of these proteins is therefore considered part of the instant disclosure for use in the present invention. Each of these fragments can be made and tested to determine whether antibodies from individuals who have SD recognize the fragments. The proteins or fragments thereof may be provided in isolated and purified form, and they may be associated with a solid matrix. The solid matrix may be present in as a component of any system that can be used for antibody detection, such as multi-well assay plates, beads, lateral flow devices or strips, or any other form or format that is suitable for keeping the proteins in a position whereby antibodies can bind to them and be detected in the method of the invention. The proteins may be covalently or non-covalently associated with the solid matrix.

[0022] The following Examples are intended to illustrate certain embodiments of the invention but are not meant to limit the invention.

Example 1

[0023] This Example demonstrates the production of autoantibodies in the sera of SD mouse models in the early stages of SD.

[0024] In order to obtain the results presented in Figure 1, sera were collected from IL-14aTG mice, NOD and C57BL/6 mice at 7 months of age. We expressed and purified SP-1, PSP and CA6 and used the purified proteins for Western blot analysis. Data shown are representative of 6 mice studied in each group. The left panel shows the serum of an IL-14aTG mouse that recognizes CA6 and PSP strongly and SP-1 weakly. The middle panel shows the same study with the serum of a C57BL/6 mouse. None of these auto-antigens were bound by C57BL/6 sera. The right panel shows the same study with the serum of an NOD mouse. Both CA6 and PSP are strongly recognized by this serum.

Example 2

[0025] This Example demonstrates that LTA is found in the salivary gland secretions of IL-14aTG mice but not in littermate controls.

[0026] In order to obtain the data results presented in Figure 2, salivary gland secretions were collected from IL-14a TG mice and various control mice at 12 months of age. Western blot assays were performed on the undiluted specimens. Lanes 1 and 2 are from IL-14a TG

mice, lanes 3 and 4 from IL-14aTG. LTA-/- mice, lanes 5 and 6 from LTA -/- mice and lanes 7 and 8 from C57BL/6 mice. We also analyzed the histology of the submandibular and parotid glands in IL-14aTG.LTA-/- mice. These are normal.

Example 3

[0027] This Example demonstrates that LTA is found in the sera of human SD patients. In order to obtain our results which are presented in Figure 3, sera were obtained from 6 normal donors (age and sex matched to 6 of the Sjogren's disease patients) and 12 patients with Sjogren's disease. Lymphotoxin levels were determined by a commercially available ELISA (R&D SYSTEMS, Inc). The difference between the serum levels of lymphotoxin between normal controls and patients with Sjogren's disease was statistically significant ($p = .0011$).

Example 4

[0028] This Example demonstrates the identification of autoantibodies in the sera and salivary glands of IL-14aTG.LTA-/- mice. The data presented in Figure 4 show that the serum of an IL-14aTG.LTA-/- mouse at 11 months of age reacts with SP-1, CA6 and PSP. The Western blot was performed as outlined in Figure 1.

Example 5

[0029] This Example demonstrates that serum obtain from human SD patients contains antibodies to murine CA6, murine PSP and murine SP-1. Western blots were performed essentially as described for Figure 1, except sera were used from patients with SD or aged matched normal controls. Five patients and two normal controls were evaluated. Data shown are from one representative patient (all five patients showed similar results) and one normal control (both normal controls showed similar results). Patients with SD but not normal controls have antibodies to PSP and SP-1. The antibody response to CA6 was stronger in SD patients than in normal controls. Thus, we have demonstrated that the presence of antibodies to CA6, PSP and CA6 can be used to diagnose SD in humans.

Claims

1. A method for determining whether a human individual has Sjögren's disease (SD) comprising

i) determining in a biological sample from the individual the presence of antibodies directed to any one of the following, salivary gland protein 1 (SP-1), parotid secretory protein (PSP),or carbonic anhydrase 6 (CA6), or determining the presence of a combination of said antibodies, and determining that the individual has SD based on the presence of the antibodies; or

determining in a biological sample obtained from the individual the absence of detection of antibodies to PSP and SP-1 and determining based on the absence of detection of the antibodies that the individual does not have SD.

2. The method of claim 1, wherein the combination of the antibodies includes antibodies to SP-1.

3. The method of claim 1, wherein the individual has early SD.

4. The method of claim 1, wherein the determining of the antibodies is performed by ELISA assay.

5. The method of claim 4, wherein the antibodies in the biological sample that are directed to SP-1 bind to murine SP-1 in the ELISA assay.

6. The method of claim 1, wherein the individual who does not have SD has a lower amount of antibodies to CA6 than an individual who has SD.

7. The method of claim 1, further comprising determining the presence or absence of autoantibodies to any one of the following, lymphotoxin (LTA), mucin 10 (submandibular gland salivary mucin), salivary amylase 1, Ro, La, muscarinic receptor 3, fodrin, IL-14, interferon-α, or combinations thereof.

8. Use in the method of claim 1 of a kit comprising salivary gland protein 1 (SP-1) or a fragment thereof that is recognized by antibodies from an individual with Sjögren's disease (SD), parotid secretory protein (PSP) or a fragment thereof that is recognized by antibodies from an individual with SD, carbonic anhydrase 6 (CA6) or a fragment thereof that is recognized by antibodies from an individual with SD, or combinations thereof.

9. The use of claim 8, wherein said SP-1 or fragment thereof that is recognized by antibodies from an individual with SD, said PSP or fragment thereof that is recognized by antibodies from an individual with SD, and said CA6 or fragment thereof that is recognized by antibodies from an individual with SD are associated with a solid matrix.

10. A use of claim 9 wherein the solid matrix is selected from multi-well assay plates, beads and lateral flow devices or strips.

11. A use of any one of claims 8 to 10 wherein said fragments that are recognized by antibodies from an individual with SD are from 9 amino acids in length up to one amino acid less than the full length of the

proteins.

12. The method of any one of claims 1 to 7 or the use of any one of claims 8 to 11 wherein the antibodies to salivary gland protein 1 (SP-1) recognizes murine SP-1.

13. The method of any one of claims 1 to 7 wherein the biological sample is a biological liquid selected from blood, serum and saliva.

14. The method of any one of claims 1 to 7, wherein the determining of the antibodies is performed by a technique selected from immunohistological techniques, western blotting, multi-well assay plates adapted for detection of the antibodies, beads adapted for detection of the antibodies, a lateral flow device or strip that is adapted for detection of the antibodies and ELISA assays.

Patentansprüche

1. Verfahren zum Bestimmen, ob ein menschliches Individuum an Sjögren-Syndrom (SS) leidet, das Folgendes umfasst:

i) Bestimmen des Vorhandenseins von Antikörpern gegen ein beliebiges der Folgenden: Speicheldrüsenprotein 1 (SP-1), sektretorisches Parotisprotein (PSP) oder Carboanhydrase 6 (CA6), oder Bestimmen des Vorhandenseins einer Kombination dieser Antikörper in einer biologischen Probe aus dem Individuum, und Bestimmen aufgrund des Vorhandenseins der Antikörper, dass das Individuum an SS leidet; oder Bestimmen des Fehlens des Nachweises von Antikörpern gegen PSP und SP-1 in einer biologischen Probe, die von dem Individuum erhalten wurde, und Bestimmen aufgrund des Fehlens des Nachweises der Antikörper, dass das Individuum nicht an SS leidet.

2. Verfahren nach Anspruch 1, wobei die Kombination der Antikörper Antikörper gegen SP-1 beinhaltet.

3. Verfahren nach Anspruch 1, wobei das Individuum an SS im Frühstadium leidet.

4. Verfahren nach Anspruch 1, wobei das Bestimmen der Antikörper mittels ELISA-Assay durchgeführt wird.

5. Verfahren nach Anspruch 4, wobei die Antikörper in der biologischen Probe, die gegen SP-1 gerichtet sind, Maus-SP-1 im ELISA-Assay binden.

6. Verfahren nach Anspruch 1, wobei das Individuum, das nicht an SS leidet, über eine geringere Menge Antikörper gegen CA6 als ein Individuum, das an SS leidet, verfügt.

7. Verfahren nach Anspruch 1, das weiterhin Folgendes umfasst: Bestimmen des Vorhandenseins oder Fehlens von Autoantikörpern gegen eine(s) der Folgenden: Lymphotoxin (LTA), Mucin 10 (Unterkieferdrüsen-Speichelmucin), Speichelamylase 1, Ro, La, Muscarinrezeptor 3, Fodrin, IL-14, Interferon-α oder Kombinationen davon.

8. Verwendung in dem Verfahren nach Anspruch 1 eines Kits, das Folgendes umfasst: Speicheldrüsenprotein 1 (SP-1) oder ein Fragment davon, das von Antikörpern aus einem Individuum, das an Sjögren-Syndrom (SS) leidet, erkannt wird, sekretorisches Parotisprotein (PSP) oder ein Fragment davon, das von Antikörpern aus einem Individuum, das an SS leidet, erkannt wird, Carboanhydrase 6 (CA6) oder ein Fragment davon, die das von Antikörpern aus einem Individuum, das an SS leidet, erkannt wird, oder Kombinationen davon.

9. Verwendung nach Anspruch 8, wobei das SP-1 oder das Fragment davon, das von Antikörpern aus einem Individuum, das an SS leidet, erkannt wird, das PSP oder das Fragment davon, das von Antikörpern aus einem Individuum, das an SS leidet, erkannt wird, und die CA-6 oder das Fragment davon, die/das von Antikörpern aus einem Individuum, das an SS leidet, erkannt wird, mit einer festen Matrix assoziiert sind.

10. Verwendung nach Anspruch 9, wobei die feste Matrix aus Multiwell-Assay-Platten, Beads und Geräten oder Streifen mit lateralem Flüssigkeitsfluss ausgewählt sind.

11. Verwendung nach einem der Ansprüche 8 bis 10, wobei die Fragmente, die von Antikörpern aus einem Individuum, das an SS leidet, erkannt werden, von 9 Aminosäuren lang bis zu eine Aminosäure weniger als die volle Länge der Proteine lang sind.

12. Verfahren nach einem der Ansprüche 1 bis 7 oder Verwendung nach einem der Ansprüche 8 bis 11, wobei die Antikörper gegen Speicheldrüsenprotein 1 (SP-1) Maus-SP-1 erkennen.

13. Verfahren nach einem der Ansprüche 1 bis 7, wobei es sich bei der biologischen Probe um eine biologische Flüssigkeit, ausgewählt aus Blut, Serum und Speichel, handelt.

14. Verfahren nach einem der Ansprüche 1 bis 7, wobei das Bestimmen der Antikörper mittels einer Technik durchgeführt wird, die aus den Folgenden ausgewählt ist: immunhistologischen Techniken, Western-

Blotting, Multiwell-Assay-Platten, die für den Nachweis der Antikörper adaptiert sind, Beads, die für den Nachweis der Antikörper adaptiert sind, einem Gerät oder einem Streifen mit lateralem Flüssigkeitsfluss, das/der für den Nachweis der Antikörper adaptiert ist, sowie ELISA-Assays.

Revendications

1. Procédé destiné à déterminer si un individu humain est atteint de la maladie de Sjögren (SD), comprenant les étapes consistant à

   i) déterminer, dans un échantillon biologique provenant de l'individu, la présence d'anticorps dirigés contre un quelconque élément parmi les suivants, la protéine 1 des glandes salivaires (SP-1), la protéine de sécrétion parotidienne (PSP) ou l'anhydrase carbonique 6 (CA6), ou bien déterminer la présence d'une combinaison desdits anticorps, et déterminer que l'individu est atteint de SD, sur la base de la présence des anticorps ; ou
   déterminer, dans un échantillon biologique obtenu à partir de l'individu, l'absence d'une détection d'anticorps contre la PSP et la SP-1 et déterminer, sur la base de l'absence d'une détection des anticorps, que l'individu n'est pas atteint de SD.

2. Procédé selon la revendication 1, dans laquelle la combinaison des anticorps comprend des anticorps contre la SP-1.

3. Procédé selon la revendication 1, dans laquelle l'individu est atteint de SD précoce.

4. Procédé selon la revendication 1, dans laquelle la détermination des anticorps est exécutée par un dosage ELISA.

5. Procédé selon la revendication 4, dans laquelle les anticorps dans l'échantillon biologique, qui sont dirigés contre la SP-1, se lient à une SP-1 murine dans le dosage ELISA.

6. Procédé selon la revendication 1, dans laquelle l'individu qui n'est pas atteint de SD a une quantité inférieure d'anticorps contre la CA6 qu'un individu atteint de SD.

7. Procédé, selon la revendication 1, comprenant en outre la détermination de la présence ou l'absence d'auto-anticorps contre un quelconque élément parmi les suivants, une lymphotoxine (LTA), la mucine 10 (mucine des glandes salivaires sous-maxillaires), l'amylase 1 de la salive, Ro, La, le récepteur muscarinique 3, la fodrine, l'IL-14, l'interféron-α, ou des combinaisons de ceux-ci.

8. Utilisation dans le procédé, selon la revendication 1, d'une trousse comprenant une protéine 1 des glandes salivaires (SP-1) ou un fragment de celle-ci qui est reconnu(e) par des anticorps provenant d'un individu atteint de la maladie de Sjögren (SD), une protéine de sécrétion parotidienne (PSP) ou un fragment de celle-ci qui est reconnu(e) par des anticorps provenant d'un individu atteint de SD, une anhydrase carbonique 6 (CA6) ou un fragment de celle-ci qui est reconnu(e) par des anticorps provenant d'un individu atteint de SD, ou des combinaisons de celles-ci.

9. Utilisation selon la revendication 8, dans laquelle ladite SP-1 ou un fragment de celle-ci qui est reconnu(e) par des anticorps provenant d'un individu atteint de SD, ladite PSP ou un fragment de celle-ci qui est reconnu(e) par des anticorps provenant d'un individu atteint de SD, et ladite CA6 ou un fragment de celle-ci qui est reconnu(e) par des anticorps provenant d'un individu atteint de SD sont associé(e)s à une matrice solide.

10. Utilisation selon la revendication 9, dans laquelle la matrice solide est choisie parmi des plaques de dosage à plusieurs puits, des billes et des dispositifs ou bandelettes à flux latéral.

11. Utilisation selon l'une quelconque des revendications 8 à 10, dans laquelle lesdits fragments qui sont reconnus par des anticorps provenant d'un individu atteint de SD ont une longueur ayant de 9 acides aminés jusqu'à un acide aminé de moins que la longueur totale des protéines.

12. Procédé, selon l'une quelconque des revendications 1 à 7, ou utilisation, selon l'une quelconque des revendications 8 à 11, dans laquelle les anticorps contre la protéine 1 des glandes salivaires (SP-1) reconnaissent la SP-1 murine.

13. Procédé selon l'une quelconque des revendications 1 à 7, dans laquelle l'échantillon biologique est un liquide biologique choisi parmi le sang, le sérum et la salive.

14. Procédé selon l'une quelconque des revendications 1 à 7, dans laquelle la détermination des anticorps est exécutée par une technique choisie parmi des techniques immuno-histologiques, un transfert de Western, des plaques de dosage à plusieurs puits adaptées à la détection des anticorps, des billes adaptées à la détection des anticorps, un dispositif ou une bandelette à flux latéral qui est adapté(e) à la détection des anticorps, ainsi que des dosages ELISA.

Figure 1

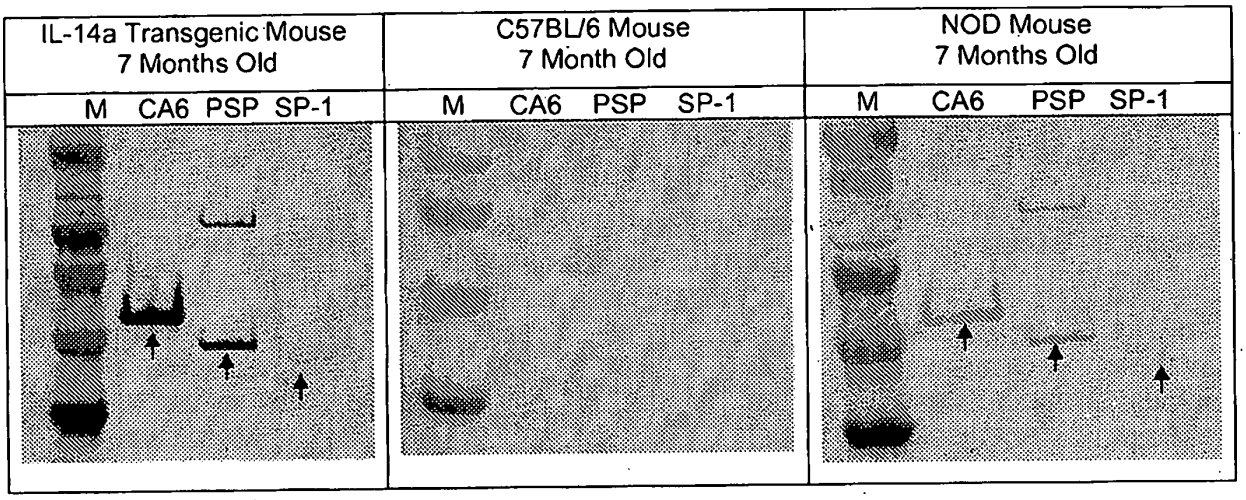


Figure 2

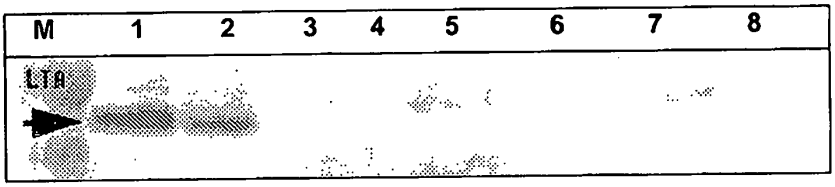


Figure 3

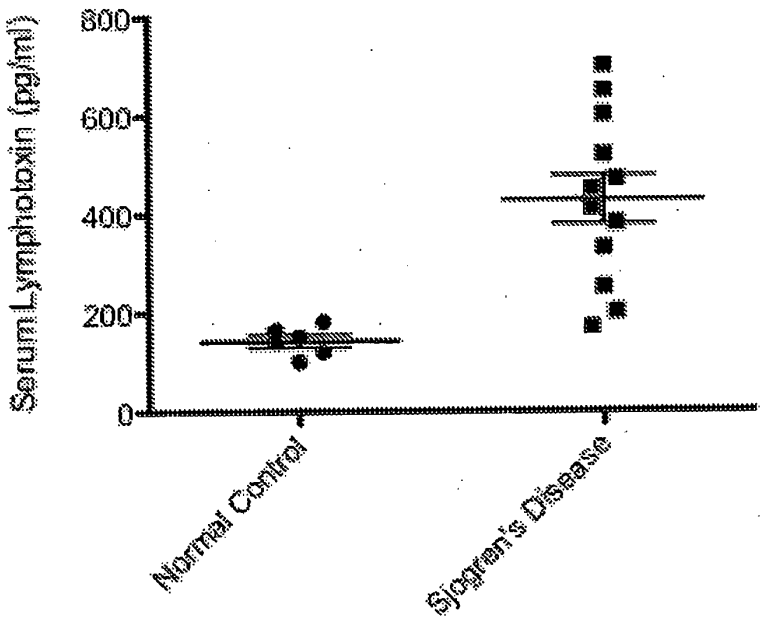


Figure 4

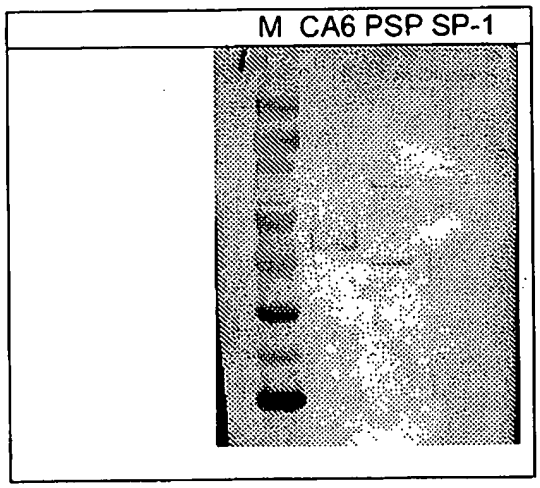
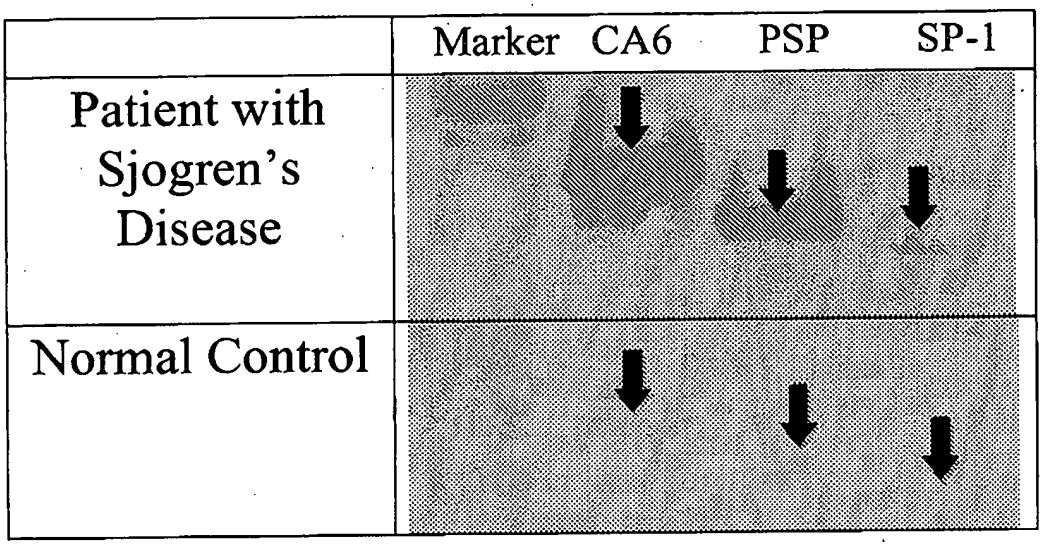


Figure 5



REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	诊断干燥综合症的方法		
公开(公告)号	EP2526422A4	公开(公告)日	2013-06-19
申请号	EP2011735215	申请日	2011-01-21
申请(专利权)人(译)	研究基金会纽约州立大学		
当前申请(专利权)人(译)	研究基金会纽约州立大学		
[标]发明人	AMBRUS JULIAN L JR SHEN LONG		
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IPC分类号	G01N33/53 G01N33/68 G01N33/564		
CPC分类号	G01N33/564 G01N2333/47 G01N2333/988 G01N2800/101 G01N33/6893 G01N2800/04		
优先权	61/297167 2010-01-21 US		
其他公开文献	EP2526422B1 EP2526422A1		
外部链接	Espacenet		

摘要(译)

提供用于确定个体是否患有干燥病（SD）的方法和组合物。 该方法需要在个体的生物样品中确定针对唾液腺蛋白1（SP-1），腮腺分泌蛋白（PSP），碳酸酐酶6（CA6）的抗体的存在，或确定抗体的组合。 确定个体是否患有SD是基于抗体的存在。 该方法提供了早期SD的检测。 还提供了用于抗体检测的试剂盒，其中包含SD患者抗体所针对的抗原。