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(54) Title: BIOMARKER FOR COMPLEX REGIONAL PAIN SYNDROME (CRPS)

(57) Abstract: The present invention relates in a first aspect to a method for diagnosing the presence or the risk of development or
the therapy control of Complex Regional Pain Syndrome (CRPS) or other types of inflammatory diseases involving joints. The
method comprises the steps of analyzing a sample from an individual to be tested for the presence of autoantibodies against
p29ING4 whereby the presence of said autoantibodies against an ING gene product, in particular, against p29ING4 is indicative for
the presence or the risk of development or the therapy control of CRPS or other types of inflammatory diseases involving joints. In
another aspect, the present invention relates to a diagnostic kit for use in the method according to the present invention. Further, the
present application relates to the use of ING gene production, in particular, of p29ING4 or immunoreactive peptides thereof in the
diagnosis, risk assessment or therapy control of CRPS or developing CRPS or other types of inflammatory diseases involving joints,
in particular, in subjects undergoing surgery of hands and feet or after bone fractures of hands and feet.

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BIOMARKER FOR COMPLEX REGIONAL PAIN SYNDROME (CRPS)

The present invention relates in a first aspect to a method for diagnosing the
presence or the risk of development or the therapy control of Complex Regional
Pain Syndrome (CRPS) and other inflammatory diseases including the joints,
e.g. spondyloarhtritis and rheumatoid arthritis. The method comprises the steps
of analyzing a sample obtained from an individual to be tested for the presence
of autoantibodies against the inhibitor of growth (ING) family proteins, in par-
ticular, the p29ING4 protein whereby the presence of said autoantibodies
against ING, in particular, p29ING4 is indicative for the presence or the risk of
development CRPS. In another aspect, the present invention relates to the use
of a diagnostic kit in the method according to the present invention. Further,
the present application relates to the use of the ING proteins, in particular, the
p29ING4 protein or immunoreactive peptides thereof in the diagnosis, risk as-
sessment or therapy control of CRPS or developing CRPS or other inflamma-
tory diseases involving the joints, in particular, in subjects undergoing sched-
uled surgery of hands and feet.

Prior art

CRPS is a syndrome characterized by a continuing (spontaneous and/or
evoked) regional pain that is seemingly disproportionate in time or degree to
the usual cause of any known trauma or other lesion. The pain is regional (not
in a specific nerve territory or dermatome) and usually has a distal predomi-
nance of abnormal sensory, motor, sudomotor, vasomotor, and/or trophic find-
ings. The syndrome shows variable progression over time.

Various clinical diagnostic criteria for CRPS exist. A recent publication of Harden, R.N. et al., Pain Med., 201 3, doi:1 0.1 11/PME.1 2033 provides an overview thereof. The clinical diagnostic criteria include continuing pain which is disproportionate to any inciting event, at least one symptom of different func-

5   tions and categories as outlined therein including sensory, vasomotor, sudomotor/edema, motor/throphic symptoms. Remarkably, the clinical diagnostics criteria for CRPS include the criteria: *"there is no other diagnosis that better explains the signs and symptoms".* Thus, it is clear, that at present diagnosis of CRPS is based on excluding other diseases and conditions, i.e. a negative di-

10   agnosis only.

CRPS can be differentiated into various subtypes, namely, CRPS I, old name "reflex sympathetic dystrophy"; CRPS II, old name "causalgia"; and CRPS-NOS (not otherwise specified) including all individuals which were not categorized in I or II and which partially meets CRPS criteria but not better ex-

15   plained by any other condition. At present, the current diagnostic guidelines distinguish between CRPS without (CRPS I) or with obvious nerve lesions (CRPS II). The main symptom of CRPS is severe neuropathic pain located deep and distally accented in the involved limb with concomitant hyperalgesia, one third of patients also suffer from allodynia (brush-evoked pain). Autonomic

20   and trophic disturbances, such as edema and swelling of the limb, skin changes, altered growth of hair and nails, and increased or decreased sweating can additionally be observed. Most patients have a weakness of the effected limb, with tremor, myoclonus, and focal dystonia frequently present.

Typically, CRPS I often occurs in the hands or feet after a minor injury,

25   while CRPS II is caused by injury to a specific nerve. It is thought that CRPS is the result from damages to the nervous system, including the nerves that control the blood vessels and sweat glands.

As CRPS is a clinical diagnosis, the characteristic clinical symptoms have to be recognized and differentiated from ordinary posttraumatic or post

30   operative findings and by the existence of other conditions or diseases. As mentioned above, at present diagnosis is based on the fact that other diseases can be excluded and *"there is no other diagnosis that better explains the signs and symptoms".* During the early phase following trauma this discrimination is

difficult because of similarities between CRPS and injury-related symptoms. Thus, the first diagnosis of CRPS is made in many cases too late, months or even years after the onset of symptoms in some patients. Evidences suggest that the earlier specific treatment is started, the more successful it is.

5   When looking into the literature, it is clear that diagnosing CRPS is difficult, but early diagnosis is very important. Typically, the doctor will take a medical history and do a physical examination. Other tests may include a method to show temperature differences and a lack of blood supply in the affected limb (thermography), as well as bone scans, nerve conduction studies

10   and x-rays. For example, clinical diagnosis of CRPS can be assisted and specified by 3-phase-bone-scintigraphy (TPBS) which is currently the gold standard of the imaging procedures. An accelerated blood flow into the affected limb combined with an increased periarticular activity during the blood pool phase and the delayed static phase are classical findings of TPBS, but the sensitivity

15   (65 %) and specificity (50 %) of TPBS for the diagnosis of CRPS I is variable throughout the literature. Aside time and effort of TPBS sensitivity and specificity of TPBS significantly decreased already three to six months after symptom onset.

       Thus, it is clear, that diagnosis is complicated by the fact that some pa-

20   tients improve without treatment and a delay in diagnosis and/or treatment for the syndrome can result in severe physical and psychological problems.

       CRPS is a frequent complication after trauma or operation with a prevalence of about 30/1 00.000. Prospective studies in patients with distal radial fracture revealed a more than 20 % incident of this complication. Hence, CRPS

25   has a relevant social impact, because it frequently causes severe long standing disability in the afflicted patients. Of note, the incidence of rheumatoid arthritis is 30/1 00.000, while for MS 4/1 00.000.

       Recently, autoimmunity against the β-2 adrenergic receptor and muscarinic-2 receptor in CRPS was described, Kohr D., et al., 201 1, Pain, 152,

30   2690-2700. Therein, identification of functionally active autoantibodies in serum samples from CRPS patients are described. In addition, antibodies directed against antigens of the autonomic nervous system, shown by reactivity of patients are sympathetic and myentheriplexus neurons as well as cholinergic neu-

roplastome cells have been detected as well. The origin of the antibodies was not known and their specificity was not established. In addition, it is not known whether the antibodies were already present before trauma. In WO 201 0/069570 peptides against autoantibodies associated with CRPS and use of these peptides have been described, based on the finding of autoantibodies against the above-cited receptor molecules.

Finally, it has been speculated that micro-RNAs (miRNAs) modulation may be used as diagnostic tool also in CRPS. Orlova IA., et al., Journal of Translational Medicine, 201 1, 9:1 95 described differences in miRNAs profiles that may be useful in patients stratification and have utility as potential bio-markers for pain.

Spondyloarthritis (Spa) also known as spondyloarthropaty or spondylitis identifies a group of diseases primarily affecting the spine and other joints. This group of diseases is also identified as seronegative spondyloarthritis. The term "seronegative" refers to the fact that usually no rheumatoid factors are present in the blood. The group of Spa can be divided into Ankylosing Spondylitis (AS), reactive arthritis and its special manifestation, Reiter Syndrome, and into psori-atic arthritis (PsA), enteropatic arthritis and undifferentiated spondyloarthritis.

Also for Rheumatoid arthritis (RA), another example of an inflammatory joint disease, diagnosis and management of said disease is important. RA is a chronic, systemic inflammatory disorder that may affects many tissues in or-gans, but principally attacks flexible joints. Until today RA diagnosis is based mainly on Y-rays imaging only.

ING4 inhibitor of growth family, member 4, (ING4), is a gene encoding a tumor protein that contains a PHD-finger, which is a common motive in proteins involved in chromatin remodelling. This protein combines TP53 and EP300/P300, a component of the histone acetyl transferase Complex, suggest-ing its involvement in TP53 dependent on regulatory pathway. The ING4 gene belongs to the ING (inhibitor of growth) tumor suppressor. An overview of the ING family is given in Gunduz, E., et al., J. Hard tissue biology, 2008, 17(1), 1-10. Thr ING family comprises at least 7 members, ING1 a, b, and c, ING 2 to 5.

Involvement of one of the ING family member ING4 in various types of tumors and cancers as well its use as a marker in tumor diagnosis and therapy

has been discussed in the literature. ING4 is also known as p29ING4. Of note, ING4 has not been discussed with respect to pain so far.

In view of the above, in particular, in view of the clinical criteria for diagnosing CRPS, in particular, CRPS 1 including the criteria of *"there is no other diagnosis that better explains the signs and symptoms of the individual"* it is clear that there is a need for providing a diagnostic tool and a method for supporting diagnosis and assessing the risk of developing CRPS including CRPS I and CRPS II, as well as a tool and method for therapy control thereof.

Further, there is a need for providing a diagnostic tool and a method for supporting diagnosis and assessing the risk of developing other inflammatory diseases involving the joints, including RA and Spa, like AS and undifferentiated spondyloarthritis.

In addition, this tool, e.g. the kit, or a method should preferably allow to differentiate between CRPS and other types of inflammatory diseases, in particular osteoarthritis, or other non-inflammatory joint diseases.

Brief description of the present invention

The present invention aims in providing a method for diagnosing the presence or the risk of development, or for therapy control of Complex Regional Pain Syndrome (CRPS), preferably of CRPS I, or other inflammatory diseases involving the joints in a subject. That is, the present inventors recognized that subjects or individuals suffering from CRPS have at least autoantibodies directed against ING family members, in particular, the molecule p29ING4, that is, the gene product of ING4. It has been recognized that the presence of autoantibodies against ING, like p29ING4, are indicative for CRPS or sensitive for the risk of development CRPS or important for treatment control of CRPS. In particular, earlier diagnosis of CRPS is possible making the laborious diagnosis redundant for excluding other diseases. Thus, a first embodiment relates to a method for diagnosing the presence or the risk of development, or for the therapy control of inflammatory disease involving the joint including RA and Spa, in particular for diagnosing the presence or the risk of development, or for the therapy control of CRPS, preferably of CRPS I, in a sub-

ject comprising

- analysing a sample obtained from a subject which may be risk of developing or may be suspected to suffering from an inflammatory disease involving the joints, in particular, CRPS, for the presence of autoantibodies against a ING family member, in particular, p29ING4,

- whereby the presence of autoantibodies against the ING family member, in particular, p29ING4 is indicative of the presence or the risk of development, or for the treatment control of the inflammatory disease involving joints, in particular, of CRPS.

Preferably, IgA and IgG autoantibodies are detected. In another preferred embodiment, the subject is a human and the autoantibodies are human autoantibodies.

Another embodiment of the present invention relates to a diagnostic kit for use in a method according to the present invention for diagnosing the presence or risk of a development as well as for treatment control of the inflammatory disease involving joints, in particular, of CRPS in a subject comprising means of determining autoantibodies against an ING gene product of the ING family, in particular, the P29ING4 protein or immunological peptides derived therefrom in a biological sample of subject to be tested and instructions how to use said test kit. Preferably, said test kit is an ELISA assay.

Brief description of the drawings

Figure 1: The figure shows the arbitrary units of the antibodies binding to p29ING4 protein in complex regional pain syndrome (CRPS, n=46), spondyloarthritis (SpA, n=46), rheumatoid arthritis (RA, n=36), Granulomatosis with Polyangitis (GPA, n=40) and blood donors (BD, n=56).

Figure 2: The figure shows the arbitrary units of the antibodies binding to p29ING4 peptide aa1-23 in complex regional pain syndrome (CRPS, n=46), spondyloarthritis (SpA, n=46), rheumatoid arthritis (RA, n=36), Granulomatosis with Polyangitis (GPA, n=40) and blood donors (BD, n=56).

Figure 3: The figure shows the arbitrary units of the antibodies binding to p29ING4 peptide aa94-1 14 in complex regional pain syndrome (CRPS, n=46), spondyloarthritis (SpA, n=46), rheumatoid arthritis (RA, n=36), Granulomatosis

with Polyangitis (GPA, n=40) and blood donors (BD, n=56).

Detailed description of the present invention

In a first aspect, the present invention relates to a method for diagnosing the presence or the risk of development, or for the therapy control of an inflammatory disease involving joints including RA and Spa, in particular, of CRPS, preferably of CRPS1, in a subject comprising

a. providing i.e. obtaining a biological sample from a subject, and

b. analysing the sample for the presence of autoantibodies against an ING gene product, in particular, against p29ING4,

whereby the presence of autoantibodies against the ING gene product, in particular, p29ING4 is indicative of the presence or the risk of development, or for the therapy control of the inflammatory disease involving joints including RA and Spa, in particular, of CRPS.

That is, the present invention is based on the observation of the present inventor that subjects afflicted with CRPS or having risk of developing CRPS as well as for therapeutic control of CRPS have autoantibodies against the ING gene product, in particular, p29ING4. The same holds true for subjects afflicted with other types of inflammatory diseases involving joint inflammation including RA and Spa.

For example, in case of Spa including AS and undifferentiated spondylarthritis, the method according to the present invention may be combined with the method as described in WO201 2/022459 including the detection of autoantibodies directed agains CD74 and/or IKBKB.

With respect to the term "autoantibody" or "autoantibodies" is meant an autoantibody that is directed against one or more of the subjects own proteins.

As used herein, the term "ING gene product" refers to a peptide, e.g. the polypeptide or the protein, of the gene encoding an ING family member including the ING1 a, ING1 b, ING1 c, ING2, ING3, ING4, and ING5 gene.

P29ING4 is the gen product of the ING4 gene. The gene product is expressed by a variety of cells. The present inventors demonstrate that the gene product of ING genes, including P29ING4 represent entities to which autoantibodies can be found in subjects afflicted with CPRS or other types of inflamma-

tory joint diseases including RA and Spa. Hence, determining the presence of autoantibodies against CRPS or other types of inflammatory diseases involving the joints including RA and Spa are indicative of the presence or the risk of de-velopment, or for the therapy control of CPRS or other types of inflammatory

5   diseases involving the joints including RA and Spa in a subject. As demon-strated in the examples, determining autoantibodies against the gene product of ING genes, in particular, against P29ING4 allow to identify individuals suffer-ing from CPRS or other types of inflammatory joint diseases including RA and Spa.

10   In a preferred embodiment, the methods allow to determine CPRS I. In another advantageous embodiment, the presence of autoantibodies against p29ING4 is determined.

The analysis and determination of the presence of autoantibodies against the ING gene product, in particular, against p29ING4 allows for the first

15   time a high-sensitive diagnostics of CRPS and a classification of CRPS from other diseases and conditions having similar symptoms.

The diagnostic method is particularly relevant for diagnosis in humans. The diagnosis may be relevant not only in case of detecting the presence of CRPS but also for assessing and diagnosing the risk of development of CRPS.

20   This might be relevant for example in case of any type of scheduled surgery of hands and feet to be conducted in said individuals. For example, in case of a necessary surgery, the individual may be tested in advance for the risk of de-velopment CPRS based on the presence of autoantibodies against an ING gene product, in particular, against p29ING4 and in case of a positive result,

25   prophylactic arrangements could be performed or the monitoring could be tightened.

That is, when having a positive result for the presence of autoantibodies against an ING gene product, in particular against p29ING4, the individual in monitored for further signs of CPRS or other types of inflammatory diseases

30   involving joints. In case of being suspected to be afflicted with CRPS or other types of inflammatory diseases involving the joints, the presence of autoanti-bodies against an ING gene product including p29ING4, gives the a artiban the

possibility to diagnose the respective disease accordingly e.g. in case of RA and Spa. Further, in therapy control, the artisan will determined the further treatment regime based on the presence of autoantibodies as defined herein. That is while in the further presence of autoantibodies a specific type of ther-

5   apy will be conducted a different kind of therapy may be admistered when the level or amount of autoantibodies change, e.g. drop or diminish.

Moreover, detecting autoantibodies against an ING gene product, in particular, against p29ING4 can be used for treatment control in case of manifest

10   CRPS or other types of inflammatory diseases involving joints including RA and Spa. That is, the presence of autoantibodies against an ING gene product, for example, against p29ING4, in particular, development of new autoantibodies and cells producing the same during therapy may be controlled accordingly.

As used herein, the terms "individual", "patient" and "subject" are used

15   interchangeably and refer to patients and subjects of humans or other mammals and include any individual it is desired to examine or treat using the methods of the present invention. However, it will be understood that "patient" does not imply that symptoms are present.

The term "biological sample" as used herein refers to a sample obtained

20   before from the subject that may be extracted, untreated, treated, isolated or concentrated therefrom. Of note, the method according to the present invention does not include the step of taking the sample from the subject. Rather, the sample is an isolated sample which may be processed further. Suitably, the biological sample is selected from any part of the patient's body, including, but

25   not limited to hair, skin, nails, tissues or body fluids, such as saliva, synovia and blood as well as liquor.

Throughout the specification, unless the context requires otherwise, the word "comprise" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step

30   or element or groups of steps of elements.

Further, the term "comprise" or "comprising" as well as "obtain" or "obtaining" include the embodiment of "consist" or "consisting of".

With the term "obtained from" is meant that a sample such as for exam-

pie, serum is isolated from or derived from a particular source of the subject including obtaining the same physically from the subject. Typically, the biological sample is obtained in advance and the method according to the present invention includes the use of said obtained and isolated biological sample pre-

5   viously isolated from the subject to be tested and physically determining the presence or absence of the autoantibodies as described herein.

As used herein, the terms "a", "an" and "the" mean "one or more" are used in this application, including the claims, unless otherwise indicated.

Unless defined otherwise, all technical and scientific terms used herein

10   have the same meaning as commonly understood to one of the ordinary skilled in the art to which the present disclosed subject matter belongs.

The terms "diagnosing" and "diagnosis" as used herein refers to methods by which a skilled artisan can estimate and even determine whether or not a subject suffering from a given disease, disorder or condition. The skilled artisan

15   makes the diagnosis on the basis of one or more diagnostic indicators, namely autoantibodies, the amount (including presence or absence) of which is indicator for the presence, severity, or absence of the condition.

Hence, "making a diagnosis" or "diagnosing", as used herein, may further include making a prognosis which can provide for predicting a clinical outcome,

20   selecting an appropriate treatment, or monitoring a current treatment and potentially change in the treatment based on the measure of a diagnostic autoantibody.

The term "determining" or "analyzing" as used herein refers to assessing the presence, absence, quantity, level or amount of the respective autoantibod-

25   ies within the subject derived sample, including qualitative or quantitative concentration levels of said substances otherwise evaluating the values or categorization of a subject clinical parameter. In particular, said terms include the physical detection or analysis, respectively. It has been determined by the inventors that autoantibodies against an ING gene product, in particular, against

30   p29ING4 can be correlated with the presence or the risk of developing CRPS, in particular, CRPS I, or other types of inflammatory diseases involving joints including RA and Spa.

In addition, in some embodiments of the presently disclosed subject mat-

ter, multiple determinations of autoantibodies over time can be made to facilitate diagnosis and/or prognosis.

As identified above, it is preferred that the isolated biological sample is selected from blood, tissue or fluid preferably selected from hair, skin, nails, saliva, synovial, liquor and blood. It is particularly preferred, that the biological sample is an isolated body fluid, preferably blood, in particular, serum.

Typically, the methods according to the present invention are in vitro methods.

The methods according to the present invention allow the diagnosis of the presence or risk of development of CRPS or other types of inflammatory joint diseases including RA and Spa, as stated above. Moreover, the method according to the present invention allows for the stratification of the therapeutic regimen of a subject afflicted with CRPS or other types of inflammatory joint diseases including RA and Spa, or being at risk of developing CRPS or other types of inflammatory joint diseases including RA and Spa. Moreover, the methods according to the present invention allows to identify the stratification of the disease, in particular, the activity of the disease and the subject afflicted with CRPS or other types of inflammatory joint diseases including RA and Spa. Based on the results obtained, the artisan will stratify the therapy, i.e. will determine the type of therapy and will apply the type of therapy accordingly.

The skilled person is well aware of useful immunodetection methods allowing analyzing the sample for the presence or absence of autoantibodies against an ING gene product, in particular, against p29ING4. For example, the biological sample obtained from the subject is contacted with an antigen, namely an ING gene product, e.g. with a p29ING4 oligo-, polypeptide or protein representing the autoantibody immunoreactive peptide, thus, allowing binding of the autoantibody to said peptide. In this connection, the terms polypeptide or protein which are used interchangeably herein, refer to a polymer of amino acids having a length of at least 50 aa. The term "oligopeptide" refers to a polymer of amino acids having a length of from 5 to 49 aa.

Contacting the chosen biological sample of the antigen under conditions effective and for a period of time sufficient to allow the formation of immune complexes, is generally a matter of adding the composition to the sample and

incubating the mixture for a period of time along enough for the autoantibodies to form immune complexes with the antigens presented. Said antigen antibody mixture can be detected by known means and methods. That is, detection of immunocomplex formation of antigen autoantibody can be achieved through

5   the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any radioactive, fluorescent, biological or enzymatic tags or labels of standard use in the art. Of course, one may find additional advantages through the use of a secondary binding ligand such as a second antibody or a biotine/avidine (streptavidine) ligand binding

10   arrangement as it is known in the art.

In some embodiments, the primary immune complex can be detected by a second binding ligand that has binding affinity for the antigen or the autoantibody presented in the sample, for example reactivity to the Fc region of the autoantibodies or having reactivity to a region of the antigen different to the

15   binding region of the autoantibody. In these cases, the second binding ligand can be linked to a detectable label or marker molecule. The second binding ligand is itself often an antibody which may thus be termed a secondary antibody. Typically, the primary immune complexes are contacted with the labelled, secondary binding ligand or antibody, under conditions effective and for a pe-

20   riod of time sufficient to allow the formation of secondary immune complexes. The secondary immune complexes are then generally washed to remove of any unbound labelled secondary antibodies or ligands, and the remaining label in the secondary immune complex is then detected.

The second binding ligand, such as an antibody, having binding activity

25   for either the antigen or autoantibody, may also be used to bind to the primary immune complexes. The second binding ligand contains an enzyme capable of processing a substrate detectable to a product and, hence, amplify a signal over time. After washing, the secondary immune complexes are contacted with substrate, permitting detection.

30   Alternatively, comparative immunodetection may be used. The skilled person is well aware of suitable methods.

It is particularly preferred that the biological sample is a body fluid, preferably blood. In particular, the biological sample is serum of the subject to be

diagnosed.

Further, in another embodiment, the methods according to the present invention allows for the stratification of the therapeutic regimen of a subject afflicted with CRPS, or being at risk of developing CRPS. That is, the present invention allows to identify the status of disease, in particular, the active state of the disease in a subject afflicted with CRPS. Thus, giving ??? the possibility to decide for the further steps of ??? including administration of suitable of pharmaceutics.

The autoantibodies to be detected may be of the IgA and/or IgG type. That is, it is possible to determine the presence of IgA and/or IgG autoantibodies in the biological sample obtained from the subject. Although it is sufficient to detect only one type of antibodies, either IgA or IgG autoantibodies, it is preferred that both IgA and IgG autoantibodies against an ING gene product, in particular, against p29ING4 are detected.

In a particular preferred embodiment, the subject is a human and the autoantibodies are human autoantibodies.

In a further embodiment, the presently disclosed subject matter provides test kits or diagnostic kits for the use in a method according to the present invention. In particular, the use of immunological kits for detecting autoantibodies in biological samples allowing diagnosis of CRPS, like CRPS I, or other types of inflammatory diseases involving joints including RA and Spa. That is, the present invention provides the use of a test kit or diagnostic kit for use in a method according to the present invention for diagnosing the presence or the risk of a development as well as for the therapy control of CRPS or other types of inflammatory diseases involving joints including RA and Spa, in a subject comprising means or determining autoantibodies against an ING gene product, in particular, against p29ING4 in a biological sample of a subject to be tested and instructions on how to use the test kit. In a preferred embodiment, said test kit is an ELISA.

Such kits can generally comprise one or more antigens, namely, oligo- or polypeptides of the ING gene product, in particular of p29ING4 that can immunoreact with the autoantibodies. Typically, the immunodetection kits will comprise in suitable container(s), one or more autoantibody immunoreactive pep-

14

tide antigens derived from the ING gene product, in particular, from p29ING4. Said antigens useful in the presently claimed methods and test kits may be the full ING gene product, in particular, the p29ING4 protein or immune reactive peptides derived therefrom.

5   For example, the immune reactive peptide is at least one peptide selected from a peptide of Seq. ID. No. 1, Seq. ID. No.2, Seq. ID. No.3, Seq. ID.No.4, and Seq. ID.No.5. It is preferred that an immune reactive peptide comprising the peptide of Seq. ID. No. 1 is used. In case of CRPS, it is also favourable to use an immune reactive peptide comprising the sequence of Seq.
10   ID. No. 5.

         In certain embodiments, the antigen can be provided bound to a solid support, such as for example a column matrix or a well of a microtiter plate, a membrane, beads, dip sticks or the like. Alternatively, the support can be provided as a separate element of the kit.

15   That is, the test kit according to the present invention for use in diagnosing CRPS or other types of inflammatory joint diseases including RA and Spa, includes beside the antigen a detection agent for the autoantibodies which may be an antibody, antibody fragment, etc. In addition, the kit may comprise more than one detection agent. If required, the kit further comprises substrate and
20   further means for allowing reaction with an enzyme used as label for the detecting agent which may be an antibody.

         The immunodetection agents of the kit can include detectable labels that are associated with or linked to the given detecting agent, in particular, the detecting antibody. Detectable labels that are associated with or attached to a
25   secondary binding ligand are also contemplated. Detectable labels include dyes, illuminescent or fluorescent molecules, biotine, radiolabels or enzymes. Typical examples for suitable labels include commonly known fluorescent molecules, like rhodamine, fluorescein, green fluorescent protein or luciferase, or alkaline phosphatase and horseradish peroxidase as examples for suitable
30   enzymes.

         Optionally, the kits further comprise positive and negative controls for verifying the results obtained when using the kit. The components of the kits can be packaged either in aqueous medium or lyophilised form and, in addition,

the kits comprise one or more containers allowing to conduct the detection. In addition, the test kit comprises instructions for use of the kit.

Moreover, the present invention relates to the use of an ING gene product, in particular, of p29ING4, immune reactive sequences or analogs thereof in
5   the diagnosis, risk assessment or therapy control of CRPS or other types of inflammatory diseases involving joints including RA and Spa.

In this connection, the term "immune reactive peptides" refers to peptide fragments of an ING gene product, in particular, of p29ING4 enabling binding with the autoantibodies derived from the subject.

10   Finally, the present invention relates to the use of peptides derived from an ING gene product, in particular, from p29ING4 including immune reactive peptides in diagnostics and/or treatment of CRPS or other types of inflammatory joint diseases involving joints including RA and Spa. That is, the use of ING gene product peptides, in particular, of p29ING4 peptides including the
15   immune reactive peptides of said molecules in the treatment of the disease identified herein allow to induce tolerance, thus, being useful in prophylaxis and therapy of CRPS. For example, the ING gene product, like p29ING4, immune reactive peptides thereof as well as analogs thereof may be used for systemic or local therapy of diseases, disorders or conditions which are associated
20   with an immunoreaction against said compounds. The administration thereof may be effected orally, parenterally or via mucosal membranes. Alternatively, the ING gene product autoantigen, in particular, the p29ING4 autoantigen may be used to eliminate autoantibodies extracorporeal^ from the subject in need thereof.

25   Alternatively, miRNA may be used as a medicament, e.g. hsa-miR-650. Of course, hsa-miR-650 may be used in diagnosis as well. That is, an increased miR-650 is indicative for the the presence or the risk of development, or for the therapy control of the complex regional pain syndrome (CRPS) or other types of inflammatory diseases involving joints, preferably of CRPS1.

30   Hence, the present invention provides pharmaceutical compositions containing ING gene product molecules, in particular, p29ING4 molecules including immune reactive peptides for use in the prophylaxis and treatment of the diseases identified herein as well as in diseases, disorders or conditions associ-

ated with an immune reaction against these compounds. The pharmaceutical composition may be provided in a suitable form. The skilled person is well aware of useful forms, dosages etc.

Examples

The following example has been included to illustrate most of the present disclosed subject matter. In the light of the present disclosure and the general level of the skilled in the art, those of skill will appreciate that the following sample are intended to be exemplary only in the numerous changes, alterations can be employed without departing from the scope of the present disclosed subject matter.

Example 1

70 sera of patients having different types of different inflammatory and rheumatic diseases were screened via protein microarray technology. A new marker of CRPS has been identified which has been evaluated further for its frequency and association accordingly.

In protein array screening, 4 of 6 patients with CRPS showed antibodies against p29ING4, 1/6 antibodies against p29ING4 and p27ING3 and 1/6 against p28ING5.

In ELISA, an evaluation was effected with sera of 46 patients with CRPS, 32 blood donors, 46 patients with spondyloarthritis (SpA), 40 patients with granulomatosis with polyangitis (GPA) and 36 having rheumatoid arthritis (RA). The patients have been well characterized with regard to the disease activity, demographic data and treatment. The sera of the patients of the controls had been stored in a -20 °C freezer until use.

ELISA:

For each of the following ELISAs we tested different conditions and each component including the plates, blocking solutions and coating solutions was tested for unspecificity.

Detection of autoantibodies against p29ING4:

In this ELISA, a recombinant full length protein of was used as the antigen. Its purity was >90% by SDS-PAGE and it was derived in an *Escherichia coli* host expression system (Source Biosciences Lifesciences, UK). For performing the ELISA tests, 96 well plates (Maxisorb, Nunc, Denmark) were coated with 1 µg human per well and phosphate saline buffer (PBS) over night. Then the plates were blocked with 300 µl PBS and 1% fat reduced milk powder (Saiiter, Obergunzburg, Germany) per well for 30 minutes at room temperature (RT). The plates were incubated with 100 µl diluted sera (1:100) in Tris-phophate buffer (TBS) with 1% fat reduced milk powder for 30 minutes at RT. After 30 minutes of incubation, the plates were washed 3 times with 300 µl 0.05% Tween-20 PBS (PBS-T) (Thermo Fisher Scientific, DK). Next, 100 µl of a secondary goat anti-human IgG (Fey fragment specific) labelled with horse radish peroxidase (HRP) (Jackson ImmunoResearch Europe Ltd.) was added in a dilution of 2:1 0000 or 2 µl in 10 ml PBS with 1% fat reduced milk powder. Instead of secondary goat anti-human IgG (Fey fragment specific) labelled with HRP (Jackson ImmunoResearch Europe Ltd.), a secondary goat anti-human IgA labelled with HRP was added in a dilution of 3:1 0000 or 3µl in 10ml PBS with 1% fat reduced milk powder for the detection of IgA autoantibodies. The plates were incubated for 30 min at room temperature and washed 3 times with PBS-T. The color reaction was performed with TMB (Thermo Fisher Scientific, DK) for up to 30 min according to the manufacturer`s instructions and the ODs were read at 450 nm in an ELISA reader. As a standard a serum of a patient with a high OD was used. The concentration of IgG antibodies against in this serum was defined as 100 U/ml.

Detection of autoantibodies binding to peptides of p29ING4:

We used the following peptides (Biomatik, Wilmington, Delaware, USA):
Amino acid sequence
(Aa)1 -23 MAAGMYLEHYLDSIENLPFELQR   (Seq. ID. No.1)
Aa24-44 FQLMRDLDQRTEDLKAEI DK (Seq. ID. No.2)

Aa45-72 LATEYMSSARSLSSEEKLALLKQIQEAYG   (Seq. ID. No.3)

Aa71 -93 AYGKCKEFGDDKVQLAMQTYEMV   (Seq. ID. No.4)

Aa94-1 14 DKHI RRLDTDLARFEADLKEK   (Seq. ID. No.5)

For performing the ELISA tests, 96 well plates (Maxisorb, Nunc, Den-

5   mark) were coated with 1 μg human synthetic peptide of p29ING4 per well and
phosphate saline buffer (PBS) over night. Then the plates were blocked with
300μl PBS and 0.2% casein (Thermo Fisher Scientific, DK) per well for 30 min-
utes at room temperature (RT). The plates were incubated with 100 μl diluted
sera (1:100) in PBS 0.2% Casein for 30 minutes at RT. After 30 minutes of in-

10   cubation, the plates were washed 3 times with 300 μl 0.05% Tween-20 PBS
(PBS-T) (Thermo Fisher Scientific, DK). Next, 100 μl of a secondary peroxi-
dase-goat anti-human IgG Fcy specific labelled with horse radish peroxidase
(HRP) (Jackson ImmunoResearch Europe Ltd.) was added in a dilution of
2:1 0000 or 2 μl in 10 ml 1% casein PBS. The plates were incubated for 30 min

15   at room temperature and washed 3 times with PBS-T. The color reaction was
performed with TMB (Thermo Fisher Scientific, DK) for up to 30 min according
to the manufacturer `s instructions and the ODs were read at 450 nm in an
ELISA reader. As a standard a serum of a patient with a high OD was used.
The concentration of IgG antibodies against each peptide in this serum was

20   defined as 100 U/ml.

The cut offs (CO) of the ELISAs were chosen by receiver operating char-
acteristic (ROC) curve analysis. We defined patients with CRPS as the fraction
of true positives out of the positives (True positive rate, TPR) and healthy do-
nors as the fraction of the false positives out of the negatives (False positive

25   rate, FPR). Each ELISA was performed with 8 negative blood donors.

Using full recombinant protein of the inhibitor of growth protein 4
(p29ING4) , the frequency of anti-IgG autoantibodies against p29ING4 was
15/46 (33%) in patients with CRPS, 14/46 (30%) in patients with SpA, 8/36
(22%) in patients with RA, 3/40 (8%) in patients with GPA, and 1/56(1 %) in

30   healthy donors.

The frequency of anti-IgA autoantibodies against p29ING4 was 18/46
(39%) in patients with CRPS, 12/46 (26%) in patients with SpA, 10/36 (28%) in
patients with RA, 2/40 (3%) in patients with GPA, 4/56 (7%) in healthy donors.

By combining both ELISAs, the frequency of autoantibodies against p29ING4 was 27/46 (59%) in patients with CRPS, 19/46 (40%) in patients with SpA, 14/36 (39%) in patients with RA, 3/40 (8%) in patients with GPA, and 4/56 (7%) in healthy donors.

By exclusion of patients with inflammatory back pain or polyarthritis, the specificity for CRPS within the groups of inflammatory joint diseases and febrile diseases increased to 100% and the sensitivity did not change.

By exclusion of patients with incidental trauma or inflammatory back pain and HLA-B27, the specificity for RA within the groups of inflammatory joint diseases and febrile diseases increased to 100% and the sensitivity did not change.

By exclusion of patients with polyarthritis and rheumatoid factors or anti-citrullinated antibodies, the specificity for RA within the groups of inflammatory joint diseases increased to 100% and the sensitivity decreased to 7/36 (19%) for IgG antibodies, 9/36 (25%) for IgA and 12/36 (33%) for IgA or IgG antibodies.

By using the detection of IgG antibodies against the peptide A1-23 of p29ING4, we could detect a similar percentage of patients as for the combined use of IgA and IgG antibodies against p29ING4. Since different patients with CRPS were analysed the sensitivity increased to 36/46 (78%) for CRPS. A similar effect could be observed for RA and SpA.

Aa1-23 peptide has a high similarity to ING1, 3 and 5, e.g peptide of p28ING5 MATAMYLEHYLDSI ENLPCELQR (Seq. ID. No. 6), or, p27ING3 LYLEDYLEMIEQLPMDL (Seq. ID. No. 7).

We detected antibodies against ING5 in 1/5 patients with CRPS by protein array screening. Therefore, CRPS or other inflammatory joint diseases might target other ING proteins as well and using these proteins as antigens might increase the sensitivity for CRPS and other inflammatory joint diseases.

Claims:

1. A method for diagnosing the presence or the risk of development, or for the therapy control of the complex regional pain syndrome (CRPS) or other types of inflammatory diseases involving joints, preferably of CRPS1, in a subject comprising

    a. providing a biological sample obtained from the subject, and

    b. analysing the sample for the presence of autoantibodies against an ING gene product, in particular, against p29ING4,

    whereby the presence of autoantibodies against the ING gene product, in particular against p29ING4, is indicative of the presence or the risk of development, or for the therapy control of CRPS or other types of inflammatory diseases involving joints.

2. The method according to claim 1, characterized in that IgA and/or IgG autoantibodies are detected.

3. The method according to any one of the preceding claims, characterized in that the subject is a human and the autoantibodies are human autoantibodies.

4. The method according to any one of the preceding claims, characterized in that detection is performed using an immunoassay, preferably with direct or indirect coupling of one reactant to a detectable marker substance.

5. The method according to claim 4, characterized in that the detection is carried out using an ELISA, RIA, multiplex immunoassay or immunofluorescence assay, western blot, line assay, dot blot assay.

6. The method according to any one of the preceding claims wherein the biological sample is an isolated sample selected from blood, tissue or fluid preferably selected from hair, skin, nails, saliva, synovia, liquor and blood.

7.   The method according to claim 6 wherein the biological sample is a body fluid, preferably blood, in particular, serum.

8.   The method according to any one of the preceding claims being an in vitro method.

9.   The method according to any one of the preceding claims allowing differentiation between CRPS and other types of diseases, in particular, arthrosis, or non-inflammatory diseases.

10.   The method according to any one of the preceding claims for the stratification of the therapeutic regimen of a subject afflicted with CRPS or other types of inflammatory diseases involving joints, or being at risk of developing CRPS or other types of inflammatory diseases involving joints.

11.   The method according to any one of the preceding claims for identifying the status of disease, in particular, the activity of the disease in a subject afflicted CRPS or other types of inflammatory diseases involving joints.

12.   The use of a diagnostic kit in a method according to any one of claims 1 to 11 for diagnosing the presence or the risk of a development as well as for therapy control of CRPS or other types of inflammatory diseases involving joints in a subject comprising means of determining autoantibodies against an ING gene product, in particular, against p29ING4, in a biological sample of a subject to be tested and instructions on how to use said test kit.

13.   The use of a diagnostic kit according to claim 12 which is an ELISA, RIA, multiplex immunoassay, immunoblot or dot blot.

14.   A use of an ING gene product, in particular, of p29ING4, or the immune reactive peptides thereof, in particular, a peptide including the amino acid sequence according to any one of Seq. ID. No. 1, 2, 3, 4, 5, 6, or 7 in the

diagnosis, risk assessment or therapy control of CRPS or developing CRPS or other types of inflammatory diseases involving joints, in particular, in subjects undergoing surgery.

5   15.   Pharmaceutical composition containing an ING gene product, in particular, p29ING4 or compounds including the immune reactive peptides thereof for use in the treatment of CRPS or other types of inflammatory diseases involving joints.
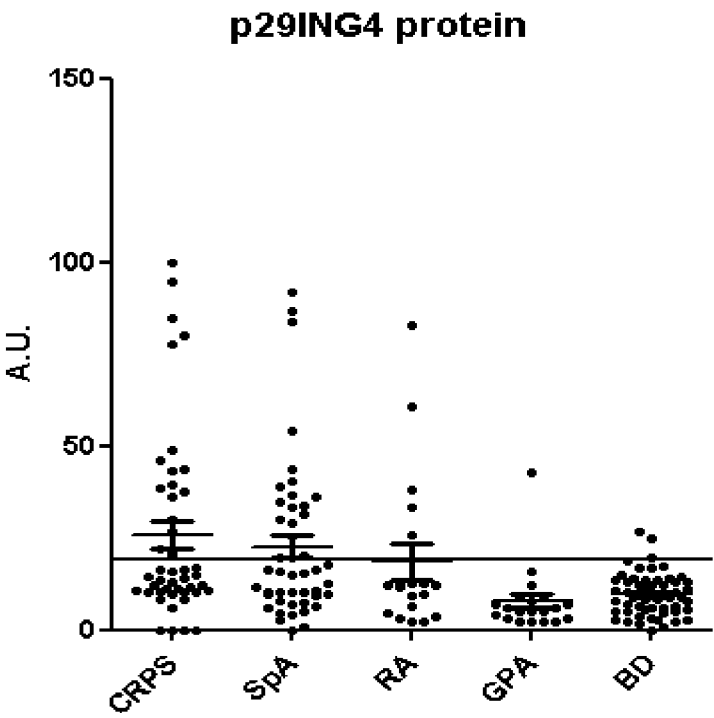
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p29ING4 protein
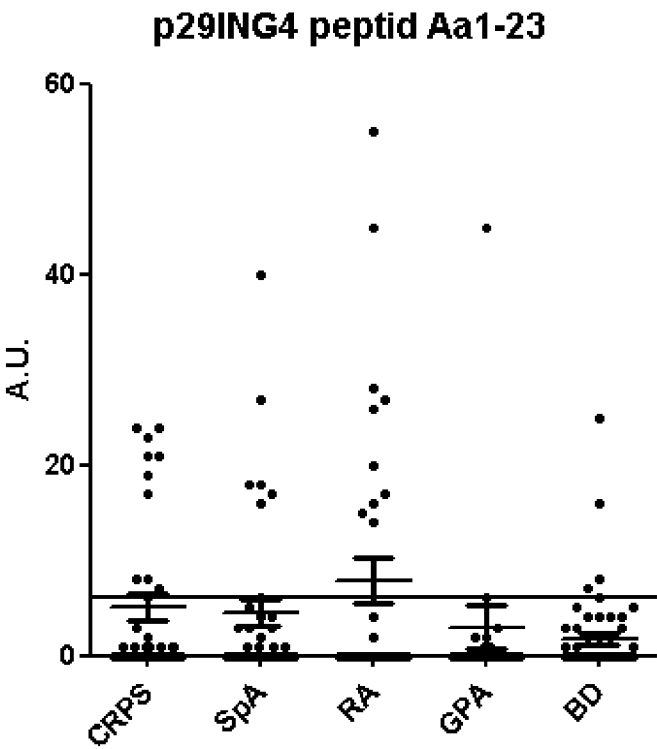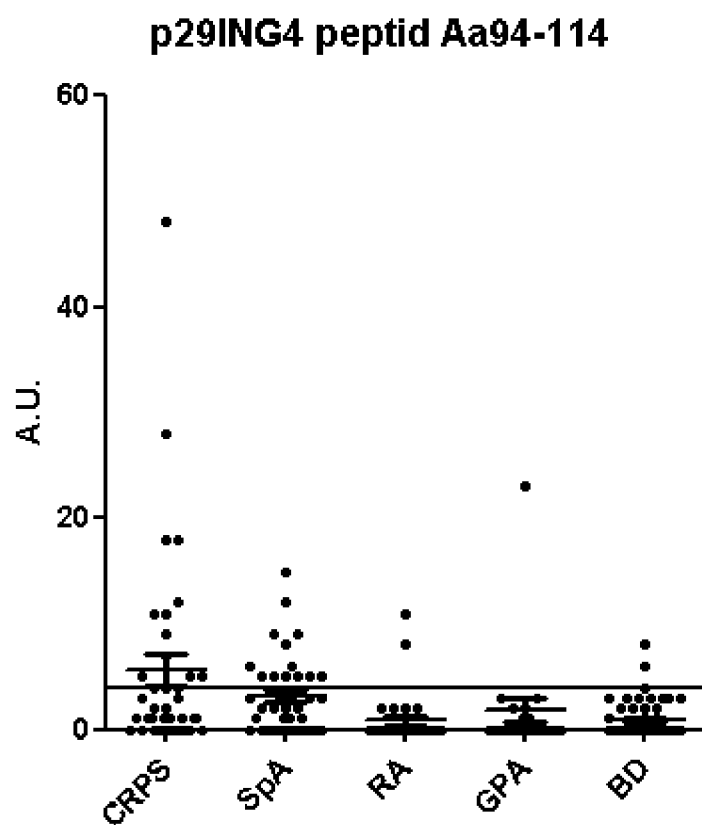

Figure 1 von 3

p29ING4 peptid Aa1-23

Figure 2 von 3

Figure 3 von 3

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/564   G01N33/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal , BIOSIS, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KOHR D ET AL: "Autoantibodies in complex regional pain syndrome bind to a differentiation-dependent neuronal surface autoantigen", PAIN, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 143, no. 3, 1 June 2009 (2009-06-01) , pages 246-251 , XP026851890, ISSN: 0304-3959 [retrieved on 2009-04-16] the whole document -----	1-15
A	EP 2 199 305 A1 (MAX DELBRUECK CT [DE]; UNIV GIESSEN JUSTUS LIEBIG [DE]) 23 June 2010 (2010-06-23) cited in the application the whole document -----	1-15
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X	Further documents are listed in the continuation of Box C.	X	See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search	Date of mailing of the international search report
27 August 2014	03/09/2014

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C(Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2005/097626 A1 (HARRIS CURTIS C [US] ET AL) 5 May 2005 (2005-05-05) the whole document In particular: paragraphs 10-12 ; claims 1-43 . -----	1-15

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
EP 2199305	AI	23-06-2010	EP	2199305	AI	23-06-2010
			WO	2010069570	A2	24-06-2010
US 2005097626	AI	05-05-2005	AU	2003209370	AI	02-09-2003
			US	2005097626	AI	05-05-2005
			US	2009081779	AI	26-03-2009
			US	2011301094	AI	08-12-2011
			US	2013177984	AI	11-07-2013
			WO	03062398	A2	31-07-2003