
Method for the detection of an autoantibody to acetylcholine receptor

The present invention relates to a diagnostically useful carrier comprising a eukaryotic cell overexpressing an adult ACh receptor or a variant thereof and a eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof, wherein the cell is fixed for immunofluorescence and a method comprising the step of contacting said carrier with a patient sample and a use of the combination of a eukaryotic cell overexpressing an adult ACh receptor or a variant thereof and a eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof for increasing the sensitivity of an assay, preferably immunofluorescence assay, for the detection of an autoantibody to ACh receptor.

In myasthenia gravis (MG), autoantibodies against the acetylcholine (ACh) receptor (AChR) of the motor end plates cause a dysfunction of neuromuscular data transmission. This leads to weakness of vital muscles, which may be fatal.

MG is divided into different forms. These encompass acquired forms of MG, either generalized MG or MG with exclusively ocular syndromes and genetic forms. The prevalence of MG is estimated to be 40 to 125 cases per 1,000,000 people in Europe and 50 to 200 in America (USA). MG can occur from early childhood to old age. Women, particularly young women of 20 to 30 years of age, and men older than 50 years of age are more likely than others to be afflicted with the disease.

If diagnosed at an early stage, life-saving treatment is possible. Therefore, the earlier an accurate diagnosis, the higher the chance that the patient can be successfully treated.

Various serological tests are available for MG diagnostics, in particular for detecting autoantibodies against AChR, for example based on ELISA, radioimmunoassay (RIA) and immunofluorescence.

While the RIA assay is the most sensitive, the use of radioactive isotopes prevents many laboratories from implementing it. Others hope that RIA tests can be replaced by sufficiently sensitive non-radioactive assays for safety reasons.

Immunofluorescence-based assays are a convenient and generally sensitive method. It has been hoped that such assays could replace the RIA, but previous studies have shown that the sensitivity of such assays leaves room for improvement.

For example George *et al.* found that autoantibodies could not be detected in one out of 13 patients (George, S., Noack, M., Vanek, M., Rentzsch, J., Röber, N., Conrad, K., Roggenbuck, D., and Küpper, J. H. (2015) Expression of nicotinic acetylcholine receptor subunits in HEp-2 cells for immunodetection of autoantibodies specificities in sera from Myasthenia gravis patients, Clin. Hamorheology and Microcirculation DOI 10.3233/CH-15199). Live cells expressing either adult or fetal ACh receptor were used for the assay and fixed after the incubation with the samples. Typical fluorescence patterns indicating the presence of an autoantibody to ACh receptor are also disclosed.

Yang *et al.*, Shi *et al.* and Leite *et al.* used an acetylcholine receptor labeled with EGFP, a 30 kD fluorescent protein, which was fused to rapsyn, a component of the complex used. ACh receptor autoantibodies were detected by detecting whether this EGFP and a fluorescent label binding to an ACh receptor autoantibody emitted colocalized fluorescence signals. (Yang, L., Maxwell, S., Leite, M. I., Waters, P., Clover, L., Fan, X., Zhang, D., Yang, C., Beeson, D., and Vincent, A. (2011) J. Neurol. Sc. 301, 71-76; Shi, Q. G., Wang, H. H., Ma, X. W., Zhang, D. Q., Yang, C. S., Shi, F. D., Yang, L. (2012) Clinical significance of detection of antibodies to fetal and adult acetylcholine receptors in myasthenia gravis, Neurosc. Bull. 28(5), 469-474; Leite, M. I., Jacob, S., Viegas, S., Cossins, J., Clover, L., Morgan, B. P., Beeson, D., Willcox, N., Vincent, A. (2008) IgG1 antibodies to acetylcholine receptors in seronegative myasthenia gravis (2008) Brain 131, 1940-1952). They used live cells fixed after incubation with a patient sample.

The problem underlying the present invention was to provide an immunofluorescence assay based on the simple detection of a fluorescent label with an increased sensitivity with the potential to replace the RIA. The assay should be in a format that allows the manufacturer to ship reagents that are ready for incubation with the samples and detection reagents, without the need for fixing directly prior to the incubation, a step that may introduce deviations that make it impossible to standardize and compare results from various laboratories.

Another problem underlying the present invention is to increase the diagnostic reliability, in particular sensitivity and/or specificity, of immunofluorescence tests.

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

In a first aspect, the problem is solved by a diagnostically useful carrier comprising a eukaryotic cell overexpressing an adult ACh receptor or a variant thereof and a eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof, wherein the cell is fixed for immunofluorescence.

In a preferred embodiment, any eukaryotic cell overexpressing an autoantigen, preferably from the group comprising an adult ACh receptor, a fetal ACh receptor and Musk, is pre-fixed.

In a preferred embodiment, the carrier further comprises a eukaryotic cell overexpressing Musk or a variant thereof.

In a preferred embodiment, the cell overexpressing an adult ACh receptor or a variant thereof and the cell overexpressing a fetal ACh receptor or a variant thereof permanently express adult and fetal ACh receptor, respectively.

In a preferred embodiment, the carrier further comprises a control, preferably a mock-transfected cell.

In a preferred embodiment, the cell is selected from the group comprising HEK293, HeLa, CHO and Jurkat cells and derivatives thereof.

In a preferred embodiment of the carrier, at least one eukaryotic cell, preferably all eukaryotic cells overexpressing an autoantigen from the group comprising adult ACh receptor, fetal ACh receptor and Musk or a variant thereof comprises or comprise one or more vectors, wherein the nucleic acid encoding the autoantigen is under the control of a promotor inducible by addition of an inducer, and wherein for each cell an additional, spatially separate eukaryotic cell is present which comprises the same vector lacking said nucleic acid or comprises the same vector, but does not express the autoantigen, preferably because of the absence of the inducer.

In a second aspect, the problem is solved by a method, preferably for the diagnosis of myasthenia gravis, LEMS and OMD, comprising the step of detecting in a sample an

autoantibody to ACh receptor, optionally in addition to detecting an autoantibody to Musk, comprising the steps:

- a) contacting the sample with the carrier according to the present invention under conditions allowing formation of a complex comprising an autoantibody from the group comprising an autoantibody to ACh receptor, preferably adult and/or fetal ACh receptor, and Musk and an autoantigen from the group comprising ACh receptor and Musk,
- b) contacting any complex formed in step b) with a fluorescent ligand to the autoantibody,
- c) removing any fluorescent ligand not bound to an autoantibody, and
- d) detecting the presence or absence of the fluorescent ligand.

In a third aspect, the problem is solved by a kit comprising the carrier according to the present invention and a ligand to an autoantibody to ACh receptor, optionally also to Musk, wherein the ligand is a fluorescent ligand, wherein any eukaryotic cell overexpressing an autoantigen is preferably pre-fixed.

In a fourth aspect, the problem is solved by a use of the combination of a eukaryotic cell overexpressing an adult ACh receptor or a variant thereof and a eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof for increasing the sensitivity of an assay, preferably immunofluorescence assay, for the detection of an autoantibody to ACh receptor.

In a preferred embodiment, the presence or absence of a fluorescent ligand binding to the autoantibody indicates whether or not the autoantibody is present in the sample.

In a preferred embodiment, the use is for diagnosing an autoimmune disease, preferably myasthenia gravis, Lambert-Eaton-Myasthenia syndrome (LEMS) and other muscular disorders OMD.

In a preferred embodiment, the eukaryotic cell expressing an adult ACh receptor or a variant thereof and the eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof and optionally the eukaryotic cell expressing Musk or a variant thereof are spatially separated.

In a preferred embodiment, the eukaryotic cell overexpressing an adult ACh receptor or a variant thereof and a eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof and optionally the eukaryotic cell expressing Musk or a variant thereof are colocalized.

In a preferred embodiment, the sample comprises antibodies from a patient, preferably blood, more preferably selected from the group comprising serum, liquor, plasma and whole blood. In a more preferred embodiment, the patient is a human patient.

The present invention is based on the inventors' surprising finding that a combination of adult and fetal AChR on the same carrier may be used to increase the sensitivity. Cells may be pre-fixed in a mass production process prior to shipping the product and incubating the carrier according to the present invention with a patient sample.

In a preferred embodiment, an ACh receptor comprises two alpha subunits and one beta and one delta subunit. In addition, the fetal ACh receptor contains the gamma subunit, whereas the adult ACh receptor contains the epsilon subunit. The complex assembles spontaneously if all the subunits required are present in a cell or *in vitro*. In a preferred embodiment, the alpha subunit is represented by SEQ ID NO1 or a variant thereof. In a preferred embodiment, the beta subunit is represented by SEQ ID NO2 or a variant thereof. In a preferred embodiment, the delta subunit is represented by SEQ ID NO3 or a variant thereof. In a preferred embodiment, the epsilon subunit is represented by SEQ ID NO4 or a variant thereof. In a preferred embodiment, the gamma subunit is represented by SEQ ID NO5 or a variant thereof.

In a preferred embodiment, the adult ACh receptor or a variant thereof and/or the fetal ACh receptor or a variant thereof are associated with rapsyn or a variant thereof. Rapsyn has been reported to aid in the assembly of ACh receptor subunits used for immunodiagnosics (Leite, M. I, Jacob, S., Viegas, S., Cossins, J., Clover, L., Morgan, B. P., Beeson, D., Willcox, N., Vincent, A. (2008) IgG1 antibodies to acetylcholine receptors in seronegative myasthenia gravis (2008) Brain 131, 1940-1952). In a preferred embodiment, rapsyn is represented by SEQ ID NO6 or a variant thereof.

In a preferred embodiment, the carrier may comprise a eukaryotic cell overexpressing Musk. In a preferred embodiment, Musk is represented by SEQ ID NO 13 or a variant thereof.

In a preferred embodiment, the diagnostically useful carrier is intended for microscopic immunofluorescence analyses. Such a carrier may be a glass slide. The cell on the glass slide may be covered with a glycerol-containing mounting buffer, and a cover glass may be placed on top. Slides with cover glasses (for example FD 1435-92, FD 1435-93, FD 1435-94 and FD 1434-90) are available from EUROIMMUN Medizinische Labordiagnostika, AG. However, any carrier compatible with microscopic analysis of the fluorescence pattern may be used. The carrier may comprise a mock-transfected cell, which has been transfected with the same vector as the cell overexpressing the autoantigen of interest, but without the nucleic acid encoding for the latter. Such mock-transfected cell may serve as a negative control. The carrier is configured for analysis using an immunofluorescence microscope.

In a preferred embodiment, a fixed eukaryotic, preferably mammalian, more preferably immortalized human cell is used. In a preferred embodiment, the term "fixed" cell, as used herein, refers to a cell that has been treated with a reactive chemical compound to the effect that the cell is no longer metabolically active, but still presents its epitopes for immunostaining with antibodies and their subsequent detection, for example by fluorescence. More preferably, the reactive chemical compound is selected from the group comprising acetone, formalin, methanol and ethanol or mixtures thereof, preferably all of them. The person skilled in the art is familiar with protocols that may be used to prepare fixed cells. Essentially, the cell which is attached to a solid support is washed by using washing buffer, followed by contacting with the reactive compound, for example immersion. Formalin or pure acetone or aqueous dilutions of the reactive chemical compound may be used.

In a preferred embodiment, the cell according to the present invention overexpresses adult ACh receptor or a variant thereof and fetal ACh receptor or a variant thereof, optionally further autoantigens such as Musk or a variant thereof. In a preferred embodiment, the term "overexpressing", as used herein, means that the cell has been transfected with a nucleic acid, either transiently or stably, the latter in the sense that the nucleic acid has been incorporated in the genome of the cell, that comprises a nucleic acid sequence encoding a polypeptide comprising the human ACh receptor or Musk or a variant thereof under the control of a promotor, preferably a strong promotor. Consequently, the transfected cell expresses more polypeptide recognized by the autoantibody than the non-transfected, same type of cell normally would, probably at least 10, 20, 30, 50, 100, 200 or 500 % more as judged by quantitative Western Blot. Preferably the non-transfected cell expresses no detectable amount of the autoantigen.

Examples of such vectors include SEQ ID NO7, SEQ ID NO8, SEQ ID NO9, SEQ ID NO10, SEQ ID NO11, SEQ ID NO12 and SEQ ID NO14.

In a preferred embodiment, the cell comprises one or more vectors comprising a nucleic acid encoding for the ACh receptor subunits under the functional control of a promoter.

In a preferred embodiment, the cell comprises the vectors represented by SEQ ID NO7 (ACh receptor alpha subunit), SEQ ID NO8 (ACh receptor beta subunit), SEQ ID NO9 (ACh receptor delta subunit) and one or both of SEQ ID NO10 (ACh receptor epsilon subunit) and SEQ ID NO11 (ACh receptor gamma subunit). In addition the cell may comprise the vector represented by SEQ ID NO12 (rapsyn) and/or the vector represented by SEQ ID NO14 (Musk).

The promoter may be an inducible promoter, which allows for the induction of expression by addition of an inducer. The person skilled in the art is familiar with protocols and vectors for transiently overexpressing a polypeptide in a eukaryotic cell, for example the pTriEx system from Novagen and with protocols and vectors for stably transfecting a eukaryotic cell, for example the pcDNATM4/TO vector system from Invitrogen.

In a preferred embodiment, the carrier may comprise a panel comprising the cell according to the invention. In addition the carrier may comprise additional panels. The panels are preferably surrounded by a hydrophobic surface.

In a preferred embodiment, the cell is bound to one or more autoantibodies each binding to an autoantigen from the group comprising fetal or adult ACh receptor or Musk, and a secondary antibody is bound to the antibody. In a more preferred embodiment, the secondary antibody recognizes IgA, IgG and IgM class antibodies or all of them, preferably class IgG antibodies. A fragment or other variant of a secondary antibody or an aptamer or other molecule with binding properties that can be engineered such that the molecule binds specifically and/or recognizes the autoantibody or IgA, IgG or IgM class antibodies may be used. For immunofluorescence analysis, the secondary antibody may comprise a detectable fluorescent ligand. In a preferred embodiment, the fluorescent ligand is a fluorescent molecule suitable for immunofluorescence staining of a eukaryotic cell, preferably selected from the group comprising fluorescein and derivatives thereof, preferably the isothiocyanate mixed isomer (FITC), tetramethylrhodamine isothiocyanate (TRITC) and derivatives thereof, rhodamine and derivatives thereof, 4,6'-Diamidino-2-phenylindole dihydrochloride (DAPI) and

derivatives thereof, 2'-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole trihydrochloride trihydrat and derivatives thereof (Hoechst 33342), and is more preferably fluorescein or a derivative thereof. Suitable fluorescent molecules are described in Martynov, V. I., Pakhomov, A. A., Popva, N. V., Dexev, I. E. and Petrenko, A. G. (2016) Synthetic Fluorophores for Visualizing Biomolecules in Living Systems, Acta Naturae, 4 (31), 33 and in Thermo Scientific Pierce fluorescent Products Guide, Thermo Scientific, 2012.

The cell according to the invention is preferably spatially separated from any of the mock-transfected cell or a cell expressing another autoantigen such that a fluorescence signal can be assigned to a specific autoantigen, for example the fetal or adult ACh receptor or Musk.

In a preferred embodiment, the adult and/or fetal ACh receptor is non-fluorescent in the sense that it is unable to emit a fluorescence signal that would be detectable in a normal fluorescence microscope used for diagnostic immunofluorescence. In other words, the ACh receptor is undetectable by immunofluorescence analysis.

In a preferred embodiment, two or more, preferably all cells on the diagnostically useful carrier, including both one or more cells overexpressing an autoantigen, preferably from the group comprising adult ACh receptor, fetal ACh receptor and Musk, as well as any cell serving as a control, are spatially separate on the carrier but close enough such that all cells can be incubated with the same sample in a one-pot reaction, ideally with a single drop of sample. For example, the Mosaik system by EUROIMMUN Medizinische Labordiagnostika AG is based on miniature panels arranged such that a number of panels, for example four or six panels, are contacted by the same drop of liquid sample.

In another preferred embodiment, a cell overexpressing adult ACh receptor and a cell overexpressing fetal ACh receptor, optionally a cell overexpressing Musk, which cells are colocalized, are used. This may be a mixture of a cell expressing an adult ACh receptor and a cell expressing a fetal ACh receptor or it may be a cell expressing both adult and fetal ACh receptor and optionally Musk.

The teachings of the present invention may not only be carried out using the polypeptides, in particular a polypeptide comprising the native sequence of a polypeptide such as the fetal or adult ACh receptor or nucleic acids having the exact sequences referred to in this application explicitly, for example by function, name, sequence or accession number, or implicitly, but also using variants of such polypeptides or nucleic acids.

In a preferred embodiment, the term "variant", as used herein, may refer to at least one fragment of the full length sequence referred to, more specifically one or more amino acid or nucleic acid sequence which is, relative to the full-length sequence, truncated at one or both termini by one or more amino acids. Such a fragment comprises or encodes for a peptide having at least 6, 7, 8, 10, 12, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 350 or 400 successive amino acids of the original sequence or a variant thereof. The total length of the variant may be at least 6, 7, 8, 9, 10, 11, 12, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 750, 1000 or more amino acids.

The term "variant" relates not only to at least one fragment, but also to a polypeptide or a fragment thereof comprising amino acid sequences that are at least 40, 50, 60, 70, 75, 80, 85, 90, 92, 94, 95, 96, 97, 98 or 99 % identical to the reference amino acid sequence referred to or the fragment thereof, wherein amino acids other than those essential for the biological activity, for example the ability of an antigen to bind to an (auto)antibody, or the fold or structure of the polypeptide are deleted or substituted and/or one or more such essential amino acids are replaced in a conservative manner and/or amino acids are added such that the biological activity of the polypeptide is preserved. The state of the art comprises various methods that may be used to align two given nucleic acid or amino acid sequences and to calculate the degree of identity, see for example Arthur Lesk (2008), Introduction to bioinformatics, Oxford University Press, 2008, 3rd edition. In a preferred embodiment, the ClustalW software (Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. Bioinformatics, 23, 2947-2948) is used using default settings.

In a preferred embodiment, the polypeptide and variants thereof may, in addition, comprise chemical modifications, for example isotopic labels or covalent modifications such as glycosylation, phosphorylation, acetylation, decarboxylation, citrullination, methylation, hydroxylation and the like. The person skilled in the art is familiar with methods to modify polypeptides. Any modification is designed such that it does not abolish the biological activity of the variant.

Moreover, variants may also be generated by N- or/and C-terminal fusion of polypeptides, fragments or variants thereof with other known polypeptides or variants thereof and comprise active portions or domains, preferably having a sequence identity of at least 70, 75, 80, 85, 90, 92, 94, 95, 96, 97, 98 or 99 % when aligned with the active portion of the reference

sequence, wherein the term "active portion", as used herein, refers to an amino acid sequence, which is less than the full length amino acid sequence or, in the case of a nucleic acid sequence, codes for less than the full length amino acid sequence, respectively, and/or is a variant of the natural sequence, but retains at least some of the biological activity. Any fusion parts are chosen such that they do not interfere with the binding reaction between autoantigen and autoantibody to be detected and do not bind to other autoantibodies and do not give a false positive or any other unwanted signal.

In a preferred embodiment, the term "variant" of a nucleic acid comprises nucleic acids the complementary strand of which hybridizes, preferably under stringent conditions, to the reference or wild type nucleic acid. Stringency of hybridization reactions is readily determinable by one of ordinary skill in the art, and in general is an empirical calculation dependent on probe length, washing temperature and salt concentration. In general longer probes require higher temperatures for proper annealing, while shorter probes less so. Hybridization generally depends on the ability of denatured DNA to reanneal to complementary strands present in an environment below their melting temperature: The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which may be used. As a result, higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperature less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel, F. M. (1995), *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc. Moreover, the person skilled in the art may follow the instructions given in the manual Boehringer Mannheim GmbH (1993) *The DIG System Users Guide for Filter Hybridization*, Boehringer Mannheim GmbH, Mannheim, Germany and in Liebl, W., Ehrmann, M., Ludwig, W., and Schleifer, K. H. (1991) *International Journal of Systematic Bacteriology* 41: 255-260 on how to identify DNA sequences by means of hybridization. In a preferred embodiment, stringent conditions are applied for any hybridization, i.e. hybridization occurs only if the probe is 70 % or more identical to the target sequence. Probes having a lower degree of identity with respect to the target sequence may hybridize, but such hybrids are unstable and will be removed in a washing step under stringent conditions, for example lowering the concentration of salt to 2 x SSC or, optionally and subsequently, to 0,5 x SSC, while the temperature is, in order of increasing preference, approximately 50°C - 68°C, approximately 52°C - 68°C, approximately 54°C - 68°C, approximately 56°C - 68°C, approximately 58°C - 68°C, approximately 60°C - 68°C, approximately 62°C - 68°C, approximately 64°C - 68°C, approximately 66°C - 68°C. In a particularly preferred embodiment, the temperature is approximately 64°C - 68°C or approximately 66°C - 68°C. It is possible to adjust the

concentration of salt to 0.2 x SSC or even 0.1 x SSC. Nucleic acid sequences having a degree of identity with respect to the reference or wild type sequence of at least 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 % may be isolated. In a preferred embodiment, the term variant of a nucleic acid sequence, as used herein, refers to any nucleic acid sequence that encodes the same amino acid sequence and variants thereof as the reference nucleic acid sequence, in line with the degeneracy of the genetic code.

The variant of the polypeptide has biological activity. In a preferred embodiment, such biological activity is the ability to bind specifically to an autoantibody binding to a mammalian ACh receptor, preferably human ACh receptor, as found in a patient suffering from an autoimmune disease associated with such autoantibody, preferably myasthenia gravis, LEMS and OMG, more preferably myasthenia gravis. For example, whether or not a variant of the polypeptide has such biological activity may be checked by determining whether or not the variant of interest binds to an autoantibody from a sample of a patient which autoantibody binds to wild type human ACh receptor, preferably as determined by indirect immunofluorescence as described in the experimental section of this application.

Various publications may aid the person skilled in the art in designing immunoreactive variants and in recognizing which variation will not abolish detection by the autoantibody. B cell epitopes have been identified on all five AChR subunits of fetal and adult forms, and the main immunogenic region is located on the acetylcholine-binding $\alpha 1$ subunit (Tzartos, S. J., Barkas, T., Cung, M.T., Mamalaki, A., Marraud, M., Orlewski, P., Papanastasiou, D., Sakarellos, C., Sakarellos-Daitsiotis, M., Tsantili, P., Tsikaris, V. Anatomy of the antigenic structure of a large membrane autoantigen, the muscle-type nicotinic acetylcholine receptor. *Immunol Rev.* 1998;163:89–120). Early studies mapping the binding sites of anti-AChR antibodies looked at binding between synthetic peptides and rat monoclonal antibodies which recognized AChR from various species, including humans. The main immunogenic region of AChR was localized to aa67-76 on the extracellular loop of the $\alpha 1$ subunit using a solid-phase radioimmunoassay where 14-20mers were incubated with the different monoclonal antibodies (Tzartos S.J., Kokla A., Walgrave S.L., Conti-Tronconi, B.M. Localization of the main immunogenic region of human muscle acetylcholine receptor to residues 67-76 of the alpha subunit. *Proc Natl Acad Sci U S A.* 1988;85:2899–2903). The importance of the $\alpha 1$ subunit was then confirmed by competition binding radioimmunoassay examining the adsorption of these monoclonal antibodies by the chosen peptide. Results of a later study indicated that interaction of the main immunogenic loop with aa1-14 on the N-terminal α -helix produces a conformational epitope on AChR that significantly augments the degree of

antibody binding (Luo J, Taylor P, Losen M, de Baets MH, Shelton GD, Lindstrom J. Main immunogenic region structure promotes binding of conformation-dependent myasthenia gravis autoantibodies, nicotinic acetylcholine receptor conformation maturation, and agonist sensitivity. *J Neurosci.* 2009;29:13898–13908). The amino acids at positions 68 and 71 appeared to be crucial contact sites, as substitutions of these residues with L-alanine nearly abolished monoclonal antibody binding (Papadouli I, Potamianos S, Hadjidakis I, Bairaktari E, Tsikaris V, Sakarellos C, Cung MT, Marraud M, Tzartos SJ. Antigenic role of single residues within the main immunogenic region of the nicotinic acetylcholine receptor. *Biochem J.* 1990;269:239–245; Wahlsten JL, Lindstrom JM, Ostlie N, Wu XD, Conti-Tronconi BM. Myasthenia gravis: effect on antibody binding of conservative substitutions of amino acid residues forming the main immunogenic region of the nicotinic acetylcholine receptor. *J Recept Res.* 1993). Autoantibodies against intracellular portions of AChRs have also been detected in MG patients, targeting a variety of subunits. Cytoplasmic epitopes reported thus far include aa373-380 of the $\alpha 1$ subunit and aa354-359 of the $\beta 1$ subunit (Tzartos, S.J., Remoundos, M. Detection of antibodies directed against the cytoplasmic region of the human acetylcholine receptor in sera from myasthenia gravis patients. *Clin Exp Immunol.* 1999; 116: 146-152).

In a more preferred embodiment, the cell is a pre-fixed cell, which term means, as used herein, that the cell has been treated with a fixing reagent such as formalin prior to incubation with the sample. In other words, the sample is absent from the cell or the carrier comprising such a cell or cells when the cell is fixed. Such a cell is metabolically inactive, but presents autoantigen epitopes such that they are reactive with autoantibodies. It is conserved and can be shipped in a kit.

In a preferred embodiment, the cell is transiently transfected, which preferably means that the cell is transfected with a vector comprising a nucleic acid encoding for an autoantigen such as fetal or adult ACh receptor or Musk, but this nucleic acid is not integrated into the genome of the cell. Consequently, the cell may lose the vector when cultivated for a prolonged time to the effect that it no longer expresses said polypeptide.

In a preferred embodiment, the cell permanently expresses an autoantigen such as fetal or adult ACh receptor or Musk, more preferably because a nucleic acid encoding for a polypeptide said autoantigen has been integrated into the genome. Consequently, the cell is likely to express the polypeptide permanently. This may also be referred to as a stable transfection or a stable expression of the polypeptide.

In a preferred embodiment, the problem is solved by a method comprising the steps of coating a diagnostically useful carrier with a eukaryotic cell overexpressing an adult ACh receptor or a variant thereof and a eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof, optionally a cell overexpressing Musk or a variant thereof, and fixing the cells for immunofluorescence.

According to the invention method, preferably for the diagnosis of myasthenia gravis, LEMS and OMD, comprising the step of detecting in a sample an autoantibody to ACh receptor, optionally in addition to an autoantibody to Musk, is provided comprising the step contacting the sample with the carrier according to the present invention under conditions allowing formation of a complex comprising an autoantibody and an autoantigen from the group comprising ACh receptor and Musk. This may be done by placing in contact with the cell a drop of the adequately diluted sample to be examined and incubating, for example for 30 minutes. PBS Tween may be used for diluting. A drop may preferably comprise 20 to 100 μ l, preferably 25 to 50 μ l. The sample may be diluted 1:1 to 1:100, preferably 1:5 to 1:20. The sample may then be removed and the cell on the carrier may be washed. PBS Tween may be used as washing buffer. If the carrier comprises more than one cell, it is preferred that the same sample is contacted with the two or more cells at the same time, more preferably in a one-pot reaction wherein the same portion of sample is in contact with two or more, preferably all cells on the carrier.

The cell and any complex formed in the previous step may then be contacted with a fluorescent ligand to the autoantibody. This can be done by placing in contact with the cell, preferably two or more or all cells on the carrier, a drop or portion of a solution comprising a ligand comprising one or more fluorescent labels. In a particularly preferred embodiment, the fluorescent label is a fluorescein derivative, preferably FITC.

Subsequently, any fluorescent ligand not bound to an autoantibody may be removed, for example by washing the carrier in an excess of washing buffer. Finally, the absence or presence of the autoantibody may be detected, for example by examining whether the carrier comprises a fluorescent pattern using an immunofluorescence microscope, for example the EUROStar Bluelight system.

In a preferred embodiment, the autoantibody, more specifically its absence or presence, is detected by detecting the presence or absence of a fluorescent ligand. There is no need to detect the colocalization of the fluorescent ligand and a fluorescent label associated with the

ACh receptor complex. Preferably the ACh receptor complex is not fluorescent or has fluorescent properties that differ from the ligand's fluorescent properties such that any fluorescent signal emitted by the AChR cannot be confused with a fluorescent signal by the fluorescent ligand.

According to the invention, a method for the detection of an autoantibody to ACh receptor, optionally in addition an autoantibody to Musk, a sample is contacted with the carrier according to the present invention under conditions allowing formation of a complex comprising an autoantibody and an autoantigen from the group comprising ACh receptor and Musk. Suitable buffers and conditions are described in the state of the art, for example George et al.. Subsequently, any excess sample is removed and the carrier may be washed. The carrier is then contacted with a fluorescent ligand to the autoantibody in the complex formed in the first step. Such a ligand may be a ligand binding to the autoantibody and carrying a fluorescent label, linked via a covalent or non-covalent bond, optionally via a linker molecule. For example, the ligand may be a secondary antibody carrying a biotin label bound to streptavidin labeled with a fluorescent label. If the fluorescent ligand comprises more than one molecule, more specifically a secondary antibody binding to the autoantibody and, via a non-covalent bond, a fluorescent molecule binding to the secondary antibody, the autoantibody may optionally first be contacted with the secondary antibody only followed, by incubation with the secondary antibody. Any excess ligand may then be separated from the carrier, for example by washing. The presence of the autoantibody is then detected by examining whether the fluorescent ligand is present or not. In a more preferred embodiment, there is no need to check the colocalization of the fluorescent ligand with another fluorescent ligand. Only one type of fluorescent label is used for the detection.

In a preferred embodiment, the fluorescent ligand binds specifically to the autoantibody, preferably in the sense that it recognizes the Ig class of the autoantibody, preferably IgG, but essentially does not bind to any other Ig classes. In a preferred embodiment, the term "binding specifically", as used herein, means that the binding is stronger than a binding reaction characterized by a dissociation constant of 1×10^{-5} M, more preferably 1×10^{-7} M, more preferably 1×10^{-8} M, more preferably 1×10^{-9} M, more preferably 1×10^{-10} M, more preferably 1×10^{-11} M, more preferably 1×10^{-12} M, as determined by surface plasmon resonance using Biacore equipment at 25 °C in PBS buffer at pH 7. The fluorescent ligand may be a monoclonal secondary or a polyclonal secondary antibody. The fluorescent ligand may be an isolated and/or purified secondary antibody. The fluorescent ligand may carry a single type of fluorescent label.

The carrier according to the present invention may be manufactured by coating a carrier such as a glass carrier with the cell or cells expressing one or more autoantigens. The cells may then be fixed. In a preferred embodiment, a fixed mammalian cell may be used. In a preferred embodiment, the term "fixed" cell, as used herein, refers to a cell that has been treated with a reactive chemical compound to the effect that the cell is no longer metabolically active, but still presents its epitopes for immunostaining with antibodies and their subsequent detection, for example by fluorescence. More preferably, the reactive chemical compound is selected from the group comprising acetone, formalin, methanol and ethanol or mixtures thereof, preferably formalin. The person skilled in the art is familiar with protocols that may be used to prepare fixed cells. Essentially, the cell which is attached to a solid support is washed by using washing buffer, followed by contacting with the reactive compound, for example immersion.

In a preferred embodiment, the term „autoantibody“, as used herein, refers to an antibody binding specifically to an endogenous molecule of the animal, preferably mammal, more preferably human, which produces said autoantibody, wherein the level of such antibody is more preferably elevated compared to the average healthy subject. In a most preferred embodiment, the autoantibody is an autoantibody binding to mammalian, preferably human ACh receptor. The autoantibody may have the sequence of an antibody's constant regions from the animal, preferably human, making it, but the variable region is able to bind specifically to the endogenous molecule of the animal, more specifically ACh receptor. In a preferred embodiment, the autoantibody is isolated and/or purified from a sample, preferably tissue, serum, plasma, blood or CSF from the animal, preferably human. The autoantibody is a polyclonal, native antibody from the animal rather than a synthetic or recombinant antibody. Preferably it is a human autoantibody.

The method according to the present invention is preferably an *in vitro* method.

According to the present invention, a kit is provided, comprising the cell or the carrier and further comprising one or more, preferably all reagents from the group comprising a ligand to an autoantibody to the ACh receptor, preferably a secondary antibody, preferably labeled with a fluorescent label, a washing buffer, a positive control, a negative control, a detergent, a cover glass, a mounting medium and a physiological salt solution, preferably PBS, or salt required to prepare it. In a preferred embodiment, the positive control is a diluted sample, preferably serum from a patient suffering from a disease associated with ACh receptor autoantibodies or a monoclonal antibody to human ACh receptor. The negative control may

be a diluted sample from a healthy subject, for example a blood donor. The kit may comprise instructions how to carry out the assay.

In a preferred embodiment, the term “immunofluorescence assay”, as used herein, means that an autoantibody is detected by fluorescence, more preferably by detection of the presence or absence of a fluorescence ligand bound to said autoantibody, while the autoantibody is bound to the autoantigen to which it binds. The autoantigen may be overexpressed by a cell and the presence or absence of the ligand may be determined by detecting, using a fluorescence microscope, whether a fluorescence signal, preferably fluorescence pattern determined by the location of the autoantigen on the cell, is present or absent.

In a preferred embodiment, any cell, method, kit, polypeptide use or carrier according to the present invention is used for the diagnosis of an autoimmune disease, preferably from the group comprising myasthenia gravis, LEMS and OMD.

In a preferred embodiment, the term „diagnosis“, as used herein, is to be used in its broadest possible sense and may refer to any kind of procedure aiming to obtain information instrumental in the assessment whether a patient suffers or is likely or more likely than the average or a comparative subject, the latter preferably having similar symptoms, to suffer from certain a disease or disorder in the past, at the time of the diagnosis or in the future, to find out how the disease is progressing or is likely to progress in the future or to evaluate the responsiveness a patient or patients in general with regard to a certain treatment, for example the administration of immunosuppressive drugs or an immunotherapeutic treatment, or to find out whether a sample is from such a patient. Such information may be used for a clinical diagnosis, but may also be obtained by an experimental and/or research laboratory for the purpose of general research, for example to determine the proportion of subjects suffering from the disease in a patient cohort or in a population. In other words, the term “diagnosis” comprises not only diagnosing, but also prognosticating and/or monitoring the course of a disease or disorder, including monitoring the response of one or more patients to the administration of a drug or candidate drug, for example to determine its efficacy. While the result may be assigned to a specific patient for clinical diagnostic applications and may be communicated to a medical doctor or institution treating said patient, this is not necessarily the case for other applications, for example in diagnostics for research purposes, where it may be sufficient to assign the results to an sample from an anonymized patient. In a preferred embodiment, the methods and products according to the present invention may be

used for interaction studies, including determining whether a drug candidate or other compound may interfere with the binding of an autoantibody to a human ACh receptor or may affect any downstream process or the strength of their binding to their target. In preferred embodiment, they may be used for monitoring the immune response, more preferably the emergence and/or titer of autoantibodies to a human autoantibody to a human ACh receptor, following the administration of an drug such as an immunosuppressive drug.

In many cases the mere detection of the autoantibody, preferably determining whether or not detectable levels of the antibody are present in the sample, is sufficient for the diagnosis. If the autoantibody can be detected, this will be information instrumental for the clinician's diagnosis and indicates an increased likelihood that the patient suffers from a disease.

The person skilled in the art will appreciate that a clinician does usually not conclude whether or not the patient suffers or is likely to suffer from a disease, condition or disorders solely on the basis of a single diagnostic parameter, but needs to take into account other aspects, for example the presence of other autoantibodies, markers, blood parameters, clinical assessment of the patient's symptoms or the results of medical imaging or other non-invasive methods such as polysomnography, to arrive at a conclusive diagnosis. See Baenkler H. W. (2012), General aspects of autoimmune diagnostics, in Renz, H., Autoimmune diagnostics, 2012, de Gruyter, page 3. The value of a diagnostic agent or method may also reside the possibility to rule out one disease, thus allowing for the indirect diagnosis of another. In a preferred embodiment, the meaning of any symptoms or diseases referred to throughout this application is in line with the person skilled in the art's understanding as of the filing date or, preferably, earliest priority date of this application as evidenced by text books and scientific publications. It should be mentioned that the inventive methods or uses or products, taken alone, cannot be used to arrive at a definite, final diagnosis.

In a preferred embodiment, the term "diagnosis" may also refer to a method or agent used to choose the most promising treatment regime for a patient. In other words, the method or agent may relate to selecting a treatment regimen for a subject. For example, the detection of autoantibodies may indicate that an immunosuppressive therapy is to be selected, which may include administrating to the patient one or more immunosuppressive drugs.

In a preferred embodiment, any information or data demonstrating the presence of absence of the autoantibody may be communicated to the patient or a medical doctor treating the

patient, preferably by telephone, in a written form or via the internet, for example as an email or text message.

In a preferred embodiment, any method or use according to the present invention may be intended for a non-diagnostic use, *i.e.* determining the presence of an autoantibody to binding to human ACh receptor for a use other than diagnosing a patient. For example, the method or use may be for testing *in vitro* the efficiency of a medical device designed to remove an autoantibody from a patient's blood, wherein the testing is performed on a liquid other than patient's blood. After the use of the medical device with a patient, its capacity to remove autoantibody may be checked by running a solution comprising antibody to human ACh receptor through the device, followed by use of the method according to the present invention to confirm that less or no antibody is in the solution that has been passed through the device, *i.e.* showing that the device has still the capacity to remove antibody from the solution.

In a preferred embodiment, the present invention provides an apparatus for analyzing a sample from a patient to detect an autoantibody against human ACh receptor, indicating an increased likelihood of a disease or of developing a disease, comprising:

- a) a carrier, which contains a means for capturing the autoantibody from the sample when the sample is contacted with the carrier, wherein the means is the cell and the carrier is the carrier according to the present invention,
- b) a detectable means capable of binding to the antibody captured by the carrier when the detectable means is contacted with the carrier, wherein the detectable means is preferably a fluorescent ligand capable of binding to the autoantibody captured on the carrier,
- c) optionally a means for removing any sample from the carrier and the detectable means, preferably by washing;
- d) a detecting device for detecting the presence or absence of the detectable means and converting the results into an electrical signal, for example a fluorescence reader connected with a software capable of recognizing a pattern characteristic of a stained cell on the carrier according to the present invention in an image of the cell taken by the fluorescence reader or camera, and

optionally a means for receiving the electronical signal from the detecting device and determining if the level of the signal is indicative of an increased likelihood of having or developing a disease, by comparing with the patterns characteristic of wild type or non-stained cells, preferably by a mock-transfected cell or cells not positively stained by an autoantibody binding to human ACh receptor on the same carrier, or an input reference value obtained with samples from healthy subjects or by comparing the level of signal obtained with one sample with the level of signal obtained with a second sample obtained at a later time point, preferably at least one month later.

Fig. 1 shows a representative Biochip mosaik comprising cells in four separate panels in each of the ten fields, more specifically fixed HEK293 cells expressing the ACh receptor comprising the epsilon subunit (upper left panel) and fixed HEK293 cells expressing the ACh receptor comprising the gamma subunit (bottom left panel). Fixed HEK293 cells transfected with the ACh receptor comprising the epsilon subunit, but without induction of expression (upper right panel) and fixed HEK293 cells transfected with the ACh receptor comprising the gamma subunit, but without induction of expression (bottom left panel) serve as negative controls.

Fig. 2 shows a fluorescence image comprising corners of the four panels following incubation with a sample comprising autoantibodies to both the ACh receptor comprising the epsilon subunit and the ACh receptor comprising the gamma subunit. The panels comprise (A) a fixed HEK293 cell expressing the ACh receptor comprising the epsilon subunit (upper left panel) and (B) a fixed HEK293 cells expressing the ACh receptor comprising the gamma subunit (bottom left panel). (C) A fixed HEK293 cell transfected with the ACh receptor comprising the epsilon subunit, but without induction of expression (upper right panel) and (D) a fixed HEK293 cells transfected with the ACh receptor comprising the gamma subunit, but without induction of expression (bottom left panel) serve as negative controls.

Sequences:

The present invention comprises a range of novel nucleic acid and polypeptide sequences, more specifically

SEQ ID NO1 CHRNA1 (Isoform 1, UniProt: P02708-2)

Mepwpllllfsllcsaglvlgsehetrlvaklfkdyssvvrpvedhrqvvvevtvglqliqlindevnqivttnvr
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SEQ ID NO2 CHRN1 (Isoform 1, UniProt: P11230-1)

Mtpgallmllgalgaplapgvrgeaegrleklfsgydssvrparevgdrvrsvglilaqlislnekdeemst
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SEQ ID NO3 CHRN2 (Isoform 1, UniProt: Q07001-1)

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SEQ ID NO4 CHRN3 (Isoform 1, UniProt: Q04844-1)

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SEQ ID NO5 CHRN4 (Isoform 1, UniProt: P07510-1)

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SEQ ID NO6 Rapsyn (Isoform 1, UniProt: Q13702-1)

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SEQ ID NO7 pTriEx-1-CHRNA1

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SEQ ID NO8 pTriEx-1-CHRN1

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SEQ ID NO13 >MuSK

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Example: Enhancing the sensitivity of an indirect immunofluorescent test for the direct detection of an autoantibody to ACh receptor

60 samples comprising an autoantibody to ACh receptor as shown by RIA (EUROIMMUN Medizinische Labordiagnostika AG, RA 1435-1, carried out according manufacturer's instructions) were subjected to immunofluorescence analysis.

A biochip mosaik comprising a panel with fixed HEK293 cells expressing the ACh receptor comprising the epsilon subunit and a panel coated with fixed HEK293 cells expressing the ACh receptor comprising the gamma subunit was prepared (**Fig. 1**). Cells were made by transfecting HEK293 cells cultured according to standard protocols with plasmids pTriEx-1-CHRNA1 (SEQ ID NO7, alpha subunit), pTriEx-1-CHRNA1 (SEQ ID NO8, beta subunit), pTriEx-1-CHRNA1 (SEQ ID NO9, delta subunit), pTriEx-1-RAPSyn (SEQ ID NO12) and either pTriEx-1-CHRNA1 (SEQ ID NO 10, epsilon subunit) or pTriEx-1-CHRNA1 (SEQ ID NO 11, gamma subunit)). The protocol provided by Invitrogen for the T-Rex™ System (Catalog number K1020-01, from 2011) was used for stable transfection. However, ExGen500 (Catalog number 12783652, Thermofisher) was used rather than Lipofectamin. according to the manufacturer's instructions followed by fixing the coated cells by immersion in formalin and followed by washing. Equal amounts of all vectors were used.

The Titerplane technology, described with suitable reagents in the manual of FA_112d-1_A_DE_C13 was subsequently used with minor modifications. Essentially human serum samples were diluted 1:10 in SAMPLE BUFFER and then 30 µl of diluted sample was placed on the biochip and incubated for 30 minutes. Unbound IgG was washed off using PBS 0.2% Tween. Any bound IgG was detected by incubating the substrate with 25-30 µl CONJUGATE_1 (biotinylated goat anti-human-IgG, EUROIMMUN), washing it with PBS-Tween and incubating the substrate with 25 µl CONJUGATE_2 (fluorescein labelled extravidin, EUROIMMUN). An example is shown in **Fig. 2**. Samples were then analyzed using a EUROStar Bluelight microscope (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck) according to the manufacturer's instructions.

Results:

55/60 samples could be shown to contain an autoantibody to AChR. 3/60 were identified as positive only when the ACh receptor comprising the epsilon subunit was used as an antigen. 2/60 could be identified as positive only when the ACh receptor comprising the gamma subunit was used.

Conclusions:

Some samples comprising autoantibodies to ACh receptor comprise autoantibodies only that are monospecific to the epsilon or gamma subunit in the assembled receptor.

Some samples could even be shown to be positive only when the ACh receptor comprising the fetal subunit was used. This is in complete contrast to George *et al.* (George, S., Noack,

M., Vanek, M., Rentzsch, J., Röber, N., Conrad, K., Roggenbuck, D., and Küpper, J. H. (2015) Expression of nicotinic acetylcholine receptor subunits in HEp-2 cells for immunodetection of autoantibodies specificities in sera from Myasthenia gravis patients, Clin. Hamorheology and Microcirculation DOI 10.3233/CH-15199).

A certain number of samples positive in the RIA could not be identified as such. However, it should also be borne in mind that the RIA has been reported to occasionally produce false-positive results (Maddison, P., Sadalage, G., Ambrose, P. A., Jacob, S., and Vincent, A. (2019) False-positive acetylcholine receptor antibody results in patients without myasthenia gravis, J. Neuroimm. 332, 69-72).

Therefore, the combination of both receptors should be used in immunofluorescence analyses, at least when the presence or absence of the autoantibodies rather than a colocalization is detected.

Claims

1. A diagnostically useful carrier comprising a eukaryotic cell overexpressing an adult ACh receptor or a variant thereof and a eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof, wherein the cell is fixed for immunofluorescence.
2. The carrier according to claim 1, wherein any eukaryotic cell overexpressing an autoantigen is pre-fixed.
3. The diagnostically useful carrier according to any of claims 1 to 2, wherein the carrier further comprises a eukaryotic cell overexpressing Musk or a variant thereof.
4. The diagnostically useful carrier according to any of claims 1 to 3, wherein the cell overexpressing an adult ACh receptor or a variant thereof and the cell overexpressing a fetal ACh receptor or a variant thereof permanently express adult and fetal ACh receptor, respectively.
5. The diagnostically useful carrier according to any of claims 1 to 4, wherein the carrier further comprises a control, preferably a mock-transfected cell.
6. The diagnostically useful carrier according to any of claims 1 to 5, wherein the cell is selected from the group comprising HEK293, HeLa, CHO and Jurkat cells and derivatives thereof.
7. The diagnostically useful carrier according to any of claims 1 to 6,

wherein at least one eukaryotic cell, preferably all eukaryotic cells overexpressing an autoantigen from the group comprising adult ACh receptor, fetal ACh receptor and Musk or a variant thereof comprises or comprise one or more vectors, wherein the nucleic acid encoding the autoantigen is under the control of a promoter inducible by addition of an inducer,

and wherein for each cell an additional spatially separate eukaryotic cell is present which comprises the same vector lacking said nucleic acid or comprises the same vector, but does not express the autoantigen, preferably because of the absence of the inducer.

8. A method for the detection of an autoantibody to ACh receptor, optionally in addition to detecting an autoantibody to Musk, comprising the steps:
 - a) contacting the sample with the carrier according to any of claims 1 to 7 under conditions allowing formation of a complex comprising an autoantibody from the group comprising an autoantibody to ACh receptor, preferably adult and/or fetal ACh receptor, and Musk and an autoantigen from the group comprising ACh receptor and Musk,
 - b) contacting any complex formed in step b) with a fluorescent ligand to the autoantibody,
 - c) removing any fluorescent ligand not bound to an autoantibody, and
 - d) detecting the presence or absence of the fluorescent ligand.
9. A kit comprising the carrier according to any of claims 1 to 7 and a ligand to an autoantibody to ACh receptor, optionally also to Musk, wherein the ligand is a fluorescent ligand, wherein any eukaryotic cell overexpressing an autoantigen is preferably pre-fixed.

10. A use of the combination of a eukaryotic cell overexpressing an adult ACh receptor or a variant thereof and a eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof for increasing the sensitivity of an immunofluorescence assay for the detection of an autoantibody to ACh receptor.
11. The use according to claim 10, wherein the presence or absence of a fluorescent ligand binding to the autoantibody indicates whether or not the autoantibody is present in a sample.
12. The carrier, method or use according to any of claims 1 to 11, wherein the eukaryotic cell expressing an adult ACh receptor or a variant thereof and the eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof and optionally the eukaryotic cell expressing Musk or a variant thereof are spatially separated.
13. The carrier, method or use according to any of claims 1 to 12, wherein the eukaryotic cell overexpressing an adult ACh receptor or a variant thereof and a eukaryotic cell overexpressing a fetal ACh receptor or a variant thereof and optionally the eukaryotic cell expressing Musk or a variant thereof are colocalized.
14. The carrier, method or use according to any of claims 1 to 13, wherein the sample comprises antibodies from a patient, preferably blood, more preferably selected from the group comprising serum, liquor, plasma and whole blood.
15. The carrier, method or use according to any of claims 1 to 14, wherein the carrier method or use is for the diagnosis of myasthenia gravis, LEMS and OMD.