



- (51) **International Patent Classification:**
C07K 16/18 (2006.01) *G01N 33/53* (2006.01)
- (21) **International Application Number:**
PCT/US2014/012801
- (22) **International Filing Date:**
23 January 2014 (23.01.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/756,318 24 January 2013 (24.01.2013) US
- (71) **Applicant:** MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH [US/US]; 200 First Street S.W., Rochester, MN 55905 (US).
- (72) **Inventors:** ASH, Peter, E.; 1505 Spindrift Circle, Neptune Beach, FL 32266-3267 (US). PETRUCELLI, Leonard; P.O. Box 1006, Ponte Vedra, FL 32009 (US). GENDRON, Tania; 13846 Atlantic Boulevard, Unit 912, Jacksonville, FL 32225 (US).
- (74) **Agent:** FINN, Patrick, J., III; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, MN 55440-1022 (US).
- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*



WO 2014/116865 A1

(54) **Title:** METHODS AND MATERIALS FOR DETECTING C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION POSITIVE FRONTOTEMPORAL LOBAR DEGENERATION OR C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION POSITIVE AMYOTROPHIC LATERAL SCLEROSIS

(57) **Abstract:** This document provides methods and materials for detecting C9ORF72 hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat expansion positive (C9⁺) frontotemporal lobar degeneration or C9⁺ amyotrophic lateral sclerosis. For example, methods and materials related to using anti-(GP)₈ (SEQ ID NO: 2) antibodies to identify mammals (e.g., humans) having C9⁺ FTLN or C9⁺ ALS are provided.

**METHODS AND MATERIALS FOR DETECTING C9ORF72
HEXANUCLEOTIDE REPEAT EXPANSION POSITIVE FRONTOTEMPORAL
LOBAR DEGENERATION OR C9ORF72 HEXANUCLEOTIDE REPEAT
EXPANSION POSITIVE AMYOTROPHIC LATERAL SCLEROSIS**

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety.
5 Said ASCII copy, created on January 22, 2014, is named SEQ.txt and is 7,275 bytes in size.

BACKGROUND

1. Technical Field

10 This document relates to methods and materials for detecting C9ORF72 hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat expansion positive (C9⁺) frontotemporal lobar degeneration (FTLD) or C9⁺ amyotrophic lateral sclerosis (ALS). For example, this document provides methods and materials related to using anti-(GP)₈ (SEQ ID NO: 2) antibodies to identify mammals (e.g., humans) having C9⁺ FTLD or C9⁺
15 ALS.

2. Background Information

FTD and ALS are both devastating neurological diseases. FTD is the second most common cause of pre-senile dementia in which degeneration of the frontal and
20 temporal lobes of the brain results in progressive changes in personality, behavior, and language with relative preservation of perception and memory (Graff-Radford and Woodruff, *Neurol.*, 27:48-57 (2007)). ALS affects 2 in 100,000 people and has traditionally been considered a disorder in which degeneration of upper and lower motor
neurons gives rise to progressive spasticity, muscle wasting, and weakness. However,
25 ALS is increasingly recognized to be a multisystem disorder with impairment of frontotemporal functions such as cognition and behavior in up to 50% of patients (Giordana *et al.*, *Neurol. Sci.*, 32:9-16 (2011); Lomen-Hoerth *et al.*, *Neurology*, 59:1077-

1079 (2003); and Phukan *et al.*, *Lancet Neurol.*, 6:994-1003 (2007)). Similarly, as many as half of FTD patients develop clinical symptoms of motor neuron dysfunction (Lomen-Hoerth *et al.*, *Neurology*, 60:1094-1097 (2002)). The concept that FTD and ALS represent a clinicopathological spectrum of disease is strongly supported by the recent
5 discovery of the transactive response DNA binding protein with a molecular weight of 43 kD (TDP-43) as the pathological protein in the vast majority of ALS cases and in the most common pathological subtype of FTD (Neumann *et al.*, *Science*, 314:130-133 (2006)), now referred to as frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP; Mackenzie *et al.*, *Acta Neuropathol.*, 117:15-18 (2009)).

10

SUMMARY

This document provides methods and materials for detecting C9⁺ FTLD or C9⁺ ALS. For example, this document provides methods and materials related to using anti-(GP)₈ (SEQ ID NO: 2) antibodies to identify mammals (e.g., humans) having C9⁺ FTLD
15 or C9⁺ ALS. As described herein, biological samples obtained from C9⁺ FTLD and C9⁺ ALS patients can contain detectable levels or elevated levels of polyGP polypeptides generated from the expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of C9ORF72 present within the C9⁺ FTLD or C9⁺ ALS patient, and antibodies generated against polyGP polypeptides (e.g., antibodies generated against C-
20 Ahx-GPGPGPGPGPGPGP-amide; SEQ ID NO:1) can be used to detect those detectable or elevated levels of polyGP polypeptides within biological samples, thereby identifying the patient as having C9⁺ FTLD or C9⁺ ALS.

Having the ability to identify mammals having C9⁺ FTLD or C9⁺ ALS using the methods and materials provided herein can allow clinicians to differentiate between those
25 patients having C9⁺ FTLD and those patients having C9⁻ FTLD and to differentiate between those patients having C9⁺ ALS and those patients having C9⁻ ALS in a quick and efficient manner without using nucleic acid amplification techniques. For example, an immunological assay involving the use of an anti-(GP)₈ (SEQ ID NO: 2) antibody and a biological sample from a mammal to be tested (e.g., a human patient suspected of
30 having FTLD or ALS) can be used to detect the presence of a detectable or elevated level of polyGP polypeptides that is indicative of C9⁺ FTLD or C9⁺ ALS, thereby indicating

that the mammal being tested has C9⁺ FTLD or C9⁺ ALS. In some cases, an immunological assay involving the use of an anti-(GP)₈ (SEQ ID NO: 2) antibody and a biological sample from a mammal to be tested (e.g., a human patient suspected of having FTLD or ALS) can be used to detect the absence of a detectable or elevated level of polyGP polypeptides that is indicative of C9⁺ FTLD or C9⁺ ALS, thereby indicating that the mammal being tested has C9⁻ FTLD or C9⁻ ALS or does not have FTLD or ALS.

C9⁻ as used herein refers to the absence of an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in the noncoding region of both C9ORF72 alleles. C9⁺ as used herein refers to the presence of an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in the noncoding region of at least one C9ORF72 allele. Healthy humans generally can have between about 2 and about 30 hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeats in the noncoding region of C9ORF72. A hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in the noncoding region of C9ORF72 is considered expanded when there are more than about 30 hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeats present. For example, a hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in the noncoding region of C9ORF72 is considered expanded when there are between about 30 and about 2000 hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeats present (e.g., between about 30 and about 1900, between about 30 and about 1800, between about 30 and about 1700, between about 30 and about 1600, between about 30 and about 1500, between about 30 and about 1400, between about 30 and about 1300, between about 30 and about 1200, between about 30 and about 1100, between about 30 and about 1000, or between about 30 and about 750 hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeats present). In some cases, a hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in the noncoding region of C9ORF72 is considered expanded when there are between about 30 and about 5000 or more hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeats present (e.g., between about 30 and about 5000, between about 30 and about 4500, between about 30 and about 4000, between about 30 and about 3500, between about 30 and about 3000, or between about 30 and about 2500 hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeats present).

This document also provides anti-(GP)₈ (SEQ ID NO: 2) antibody preparations. As described herein, an anti-(GP)₈ (SEQ ID NO: 2) antibody preparation can be a

preparation that includes one or more anti-(GP)₈ (SEQ ID NO: 2) antibodies having the ability to bind a (GP)₈ (SEQ ID NO: 2) polypeptide (GPGPGPGPGP-GPGPGP; SEQ ID NO:2). In some cases, an anti-(GP)₈ (SEQ ID NO: 2) antibody of an anti-(GP)₈ (SEQ ID NO: 2) antibody preparation provided herein can bind to a (GP)₈ (SEQ ID NO: 2) polypeptide with no detectable binding to a (PGP)₈ (SEQ ID NO: 4) polypeptide or to a collagen polypeptide. For example, an anti-(GP)₈ (SEQ ID NO: 2) antibody preparation provided herein can include anti-(GP)₈ (SEQ ID NO: 2) antibodies that bind to a (GP)₈ (SEQ ID NO: 2) polypeptide with no detectable binding to a (PGP)₈ (SEQ ID NO: 4) polypeptide. Examples of anti-(GP)₈ (SEQ ID NO: 2) antibodies having the ability to bind to a (GP)₈ (SEQ ID NO: 2) polypeptide with no detectable binding to a (PGP)₈ (SEQ ID NO: 4) polypeptide include, without limitation, antibodies generated against C-Ahx-GPGPGPGPGPGPGP-amide (SEQ ID NO:1). Anti-(GP)₈ (SEQ ID NO: 2) antibody preparations provided herein can be used to identify mammals having C9⁺ FTLN or C9⁺ ALS. For example, anti-(GP)₈ (SEQ ID NO: 2) antibody preparations provided herein can be used to identify humans having C9⁺ FTLN or can be used to bind to polypeptides having eight or more (e.g., from 8 to 2000, from 8 to 1900, from 8 to 1800, from 8 to 1700, from 8 to 1600, from 8 to 1500, from 8 to 1000, from 8 to 750, from 8 to 500, from 8 to 250, from 8 to 200, from 8 to 150, from 8 to 100, from 8 to 75, from 8 to 50, or from 8 to 25) GP repeats. In some cases, an anti-(GP)₈ (SEQ ID NO: 2) antibody preparations provided herein can be used to detect a polypeptide having a (GP)₈₋₂₀₀₀ amino acid sequence.

In general, one aspect of this document features a method for identifying a mammal having an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of at least one C9ORF72 allele. The method comprises, or consists essentially of, (a) contacting a biological sample obtained from the mammal with an anti-polyGP antibody under conditions wherein a polyGP polypeptide present within the biological sample and the anti-polyGP antibody form a polyGP polypeptide/anti-polyGP antibody complex, (b) detecting the polyGP polypeptide/anti-polyGP antibody complex, and (c) classifying the mammal as having the expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat. The mammal can be a human. The biological sample can be a cerebrospinal fluid sample. The biological sample can be a blood sample. The binding

affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10⁶ mol⁻¹ and 10¹² mol⁻¹. The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10⁶ mol⁻¹ and 10¹² mol⁻¹, and the binding affinity of the anti-polyGP antibody for a (PGP)₈ (SEQ ID NO: 4) polypeptide can be less than 10³ mol⁻¹.

In another aspect, this document features a method for identifying a human as having a C9⁺ neurological condition. The method comprises, or consists essentially of, (a) contacting a biological sample obtained from a human with an anti-polyGP antibody under conditions wherein a polyGP polypeptide present within the biological sample and the anti-polyGP antibody form a polyGP polypeptide/anti-polyGP antibody complex, (b) detecting the polyGP polypeptide/anti-polyGP antibody complex, and (c) classifying the human as having a C9⁺ neurological condition. The biological sample can be a cerebrospinal fluid sample. The biological sample can be a blood sample. The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10⁶ mol⁻¹ and 10¹² mol⁻¹. The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10⁶ mol⁻¹ and 10¹² mol⁻¹, and the binding affinity of the anti-polyGP antibody for a (PGP)₈ (SEQ ID NO: 4) polypeptide can be less than 10³ mol⁻¹. The C9⁺ neurological condition can be C9⁺ FTLN. The C9⁺ neurological condition can be C9⁺ ALS.

In another aspect, this document features an anti-polyGP antibody. The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10⁶ mol⁻¹ and 10¹² mol⁻¹. The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10⁶ mol⁻¹ and 10¹² mol⁻¹, and wherein the binding affinity of the anti-polyGP antibody for a (PGP)₈ (SEQ ID NO: 4) polypeptide can be less than 10³ mol⁻¹. The anti-polyGP antibody can be an anti-(GP)₈ (SEQ ID NO: 2) antibody.

In another aspect, this document features an antibody preparation comprising an anti-polyGP antibody. The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10⁶ mol⁻¹ and 10¹² mol⁻¹. The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10⁶ mol⁻¹ and 10¹² mol⁻¹, and the binding affinity of the anti-polyGP antibody for a (PGP)₈

(SEQ ID NO: 4) polypeptide can be less than 10^3 mol^{-1} . The anti-polyGP antibody can be an anti-(GP)₈ (SEQ ID NO: 2) antibody.

In another aspect, this document features a method for detecting a polyGP polypeptide present within the sample. The method comprises, or consists essentially of, (a) contacting the biological sample with an anti-polyGP antibody under conditions wherein the polyGP polypeptide present within the sample and the anti-polyGP antibody form a polyGP polypeptide/anti-polyGP antibody complex, and (b) detecting the polyGP polypeptide/anti-polyGP antibody complex, thereby detecting the presence of the polyGP polypeptide within the sample. The biological sample can be a cerebrospinal fluid sample. The biological sample can be a blood sample. The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10^6 mol^{-1} and 10^{12} mol^{-1} . The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10^6 mol^{-1} and 10^{12} mol^{-1} , and the binding affinity of the anti-polyGP antibody for a (PGP)₈ polypeptide can be less than 10^3 mol^{-1} . The anti-polyGP antibody can be a monoclonal antibody.

In another aspect, this document features a method for detecting the presence or absence of a polyGP polypeptide within the sample. The method comprises, or consists essentially of, (a) contacting the biological sample with an anti-polyGP antibody under conditions wherein the polyGP polypeptide, if present within the sample, and the anti-polyGP antibody form a polyGP polypeptide/anti-polyGP antibody complex, and (b) determining the presence or absence of the polyGP polypeptide/anti-polyGP antibody complex from step (a), wherein the presence of the complex indicates the presence of the polyGP polypeptide within the sample, and wherein the absence of the complex indicates the absence of the polyGP polypeptide within the sample. The biological sample can be a cerebrospinal fluid sample. The biological sample can be a blood sample. The binding affinity of the anti-polyGP antibody for a (GP)₈ polypeptide can be between 10^6 mol^{-1} and 10^{12} mol^{-1} . The binding affinity of the anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide can be between 10^6 mol^{-1} and 10^{12} mol^{-1} , and the binding affinity of the anti-polyGP antibody for a (PGP)₈ (SEQ ID NO: 4) polypeptide can be less than 10^3 mol^{-1} . The anti-polyGP antibody can be a monoclonal antibody.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 is a schematic of translation products of the three alternate frames of the sense RNA transcript generated from the expanded C9ORF72 GGGGCC (SEQ ID NO: 3) repeat that would generate repeating dipeptides of (Glycine-Alanine)_n, (Glycine-Proline)_n, and (Glycine-Arginine)_n, and the translation products of the three alternate frames of the anti-sense RNA GGCCCC (SEQ ID NO: 5) repeat that would generate repeating dipeptides of (Proline-Alanine)_n, (Proline-Glycine)_n, and (Proline-Arginine)_n. Figure 1 discloses SEQ ID NOS 14-21, respectively, in order of appearance.

Figure 2A is a photograph of a dot blot demonstrating that MC-001 antibody (a polyclonal antibody raised in rabbits against a mixture of C-Ahx-GAGAGAGAGAGAGAGA-amide (SEQ ID NO: 6), C-Ahx-GPGPGPGPGPGPGP-amide (SEQ ID NO: 1), and C-Ahx-GRGRGRGRGRGRGR-amide (SEQ ID NO: 7)) exhibits high affinity for the (GP)₈ (SEQ ID NO: 2) polypeptide as compared to a (GA)₈ (SEQ ID NO: 8) polypeptide, a (GR)₈ (SEQ ID NO: 9) polypeptide, a (GK)₈ (SEQ ID NO: 10) polypeptide, or a (GX)₈ (SEQ ID NO: 11) polypeptide ("X" represents a random amino acid that is not glycine, alanine, proline, arginine, cysteine, valine, or lysine). The unconjugated (GP)₈ (SEQ ID NO: 2) polypeptides, (GA)₈ (SEQ ID NO: 8) polypeptides,

(GR)₈ (SEQ ID NO: 9) polypeptides, (GK)₈ (SEQ ID NO: 10) polypeptides, and (GX)₈ (SEQ ID NO: 11) polypeptides were dotted onto a nitrocellulose membrane at the amounts shown and probed with 100 ng/mL of the MC-001 antibody. Figure 2B is a graph plotting the binding signal (relative absorbance units; A.U.) of 500 ng/mL of the MC-001 antibody to unconjugated (GP)₈ (SEQ ID NO: 2) polypeptides, (GA)₈ (SEQ ID NO: 8) polypeptides, (GR)₈ (SEQ ID NO: 9) polypeptides, (GK)₈ (SEQ ID NO: 10) polypeptides, and (GX)₈ (SEQ ID NO: 11) polypeptides as detected using the Meso Scale Discovery (MSD™) platform.

Figure 3 contains photographs of the indicated tissues from C9⁺ FTLD (C9ORF72+) and C9⁻ FTLD (C9ORF72-) patients stained with the MC-001 antibody. The MC-001 antibody detected inclusions in cerebellar granule and Purkinje cells throughout hippocampus proper and dentate fascia and in the frontal cortex of C9⁺ FTLD patients, but not in C9⁻ FTLD patients.

Figures 4A-F are photographs of immunohistochemistry using the MC-001 antibody to stain tissue from patients with Alzheimer's disease (A, B), spinocerebellar ataxia type 3 (C, D), and Huntington's disease (E, F). Dentate fascia (A) and hippocampus proper (B) of the Alzheimer's disease patient exhibited no MC-001 antibody immunoreactivity. Pontine nucleus (C) and locus ceruleus (D) of the spinocerebellar ataxia type 3 patient exhibited non-specific diffuse nuclear staining and melanin in the cytoplasm (a natural pigment of the neurons of this nucleus). Basal forebrain nucleus of Meynert (E) and putamen (F) of the Huntington's disease patient exhibited non-specific diffuse nuclear staining.

Figure 5A is a photograph of an immunoblot of a urea fraction from a sequential extraction of cerebellar tissue using 100 ng/mL of the MC-001 antibody. Figure 5B is a photograph of a dot blot of 2 μL urea fractions blotted onto a nitrocellulose membrane and probed with 50 ng/mL of the MC-001 antibody. Figure 5C is a graph plotting the levels of insoluble material detected in the urea fraction using the MSD™ platform and the MC-001 antibody (t-test; P=0.0004).

Figure 6 is a bar graph plotting the normalized binding signal of the MC-001 antibody to poly(GP) polypeptides present in cerebral spinal fluid (CSF) from control patients (CLT), C9⁻ ALS patients, and a C9⁺ ALS patient. To measure anti-poly(GP)

polypeptide immunoreactivity in CSF obtained from healthy controls (CTL, n=2), C9-ALS patients (ALS, n=6), and an ALS patient with the C9ORF72 mutation (C9⁺ ALS, n=1), 90 μ L of CSF was adsorbed to carbon electrodes at the bottom of a 96-well MSDTM assay plate. After immobilization and blocking, the MC-001 antibody in combination
5 with a Sulfo-tagged rabbit secondary antibody was added to wells. Immunoreactivity was measured by adding MSDTM 1x Read Buffer to wells and reading the light emission at 620 nm after electrochemical stimulation using the MSDTM Sector Imager 2400.

Figure 7 is a bar graph plotting the binding signals of monoclonal antibody preparations made to detect poly(GP) polypeptides from three different clones to the
10 indicated polypeptides.

DETAILED DESCRIPTION

This document provides methods and materials for detecting C9⁺ FTLD or C9⁺ ALS. For example, this document provides methods and materials related to using anti-
15 (GP)₈ (SEQ ID NO: 2) antibodies to identify mammals (e.g., humans) having C9⁺ FTLD or C9⁺ ALS. As described herein, biological samples obtained from C9⁺ FTLD and C9⁺ ALS patients can contain detectable levels or elevated levels of polyGP polypeptides generated from the expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of C9ORF72 present within the C9⁺ FTLD or C9⁺ ALS patient, and
20 antibodies generated against polyGP polypeptides (e.g., antibodies generated against C-Ahx-GPGPGPGPGPGPGP-amide; SEQ ID NO:1) can be used to detect those detectable or elevated levels of polyGP polypeptides within biological samples, thereby identifying the patient as having C9⁺ FTLD or C9⁺ ALS.

Any appropriate mammal can be assessed for C9⁺ FTLD or C9⁺ ALS or can be
25 assessed for the presence of an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of C9ORF72 as described herein. For example, humans, non-human primates, monkeys, horses, cows, sheep, pigs, dogs, and cats can be assessed for the presence of an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of C9ORF72 using anti-polyGP polypeptide antibodies such as the
30 MC001 antibody. In some cases, a human diagnosed as having FTLD or ALS can be assessed for the presence of an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3)

repeat in a noncoding region of C9ORF72 using an anti-polyGP polypeptide antibody as described herein.

When assessing a mammal for detectable levels or elevated levels of polyGP polypeptides generated from an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of C9ORF72, any appropriate sample can be evaluated. For example, tissue samples, cerebrospinal fluid samples, or blood samples can be obtained from a mammal and tested to determine the level of polyGP polypeptides.

Any appropriate method can be used to assess the level of polyGP polypeptides within a sample. For example, immunological assays such as Western blots, FACS analyses, ELISAs, and RIAs can be used to determine whether or not a sample contains a detectable level or elevated level of polyGP polypeptides generated from an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of C9ORF72.

In immunological assays, an antibody having specific binding affinity for a polyGP polypeptide (e.g., a (GP)₈ (SEQ ID NO: 2) polypeptide or the MC001 antibody) or a secondary antibody that binds to such an antibody can be labeled, either directly or indirectly. Suitable labels include, without limitation, radionuclides (e.g., ¹²⁵I, ¹³¹I, ³⁵S, ³H, ³²P, ³³P, or ¹⁴C), fluorescent moieties (e.g., fluorescein, FITC, PerCP, rhodamine, or PE), luminescent moieties (e.g., Qdot™ nanoparticles supplied by Invitrogen (Carlsbad, CA)), compounds that absorb light of a defined wavelength, or enzymes (e.g., alkaline phosphatase or horseradish peroxidase). Antibodies can be indirectly labeled by conjugation with biotin and then detected with avidin or streptavidin labeled with a molecule described above. Methods of detecting or quantifying a label depend on the nature of the label and are known in the art. Examples of detectors include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers. Combinations of these approaches (including “multi-layer” assays) familiar to those in the art can be used to enhance the sensitivity of assays.

Immunological assays for detecting polyGP polypeptides generated from an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of C9ORF72 can be performed in a variety of formats including, without limitation, sandwich assays, competition assays (competitive RIA), or bridge immunoassays. See,

for example, U.S. Patent Nos. 5,296,347; 4,233,402; 4,098,876; and 4,034,074. Methods of detecting polyGP polypeptides generated from an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of C9ORF72 generally include contacting a biological sample (e.g., a cerebrospinal fluid sample or blood sample) with an antibody that binds to a polyGP polypeptide (e.g., a (GP)₈ (SEQ ID NO: 2) polypeptide) and detecting binding of the polyGP polypeptide to the antibody. For example, an antibody having specific binding affinity for a polyGP polypeptide (e.g., a (GP)₈ (SEQ ID NO: 2) polypeptide) can be immobilized on a solid substrate by any of a variety of methods known in the art and then exposed to the biological sample. Binding of the polyGP polypeptide to the antibody on the solid substrate can be detected by exploiting the phenomenon of surface plasmon resonance, which results in a change in the intensity of surface plasmon resonance upon binding that can be detected qualitatively or quantitatively by an appropriate instrument, e.g., a Biacore apparatus (Biacore International AB, Rapskatan, Sweden). In some cases, the antibody can be labeled and detected as described above. A standard curve using known quantities of a polyGP polypeptide can be generated to aid in the quantitation of the levels of the polyGP polypeptide within a sample being tested.

The term “antibody” as used herein refers to intact antibodies as well as antibody fragments that retain some ability to bind an epitope. Such fragments include, without limitation, Fab, F(ab')₂, and Fv antibody fragments. The term “epitope” refers to an antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules (e.g., amino acid or sugar residues) and usually have specific three dimensional structural characteristics as well as specific charge characteristics.

The antibodies provided herein can be any antibody (e.g., a monoclonal or polyclonal antibody) having specific binding affinity for a polyGP polypeptide (e.g., a (GP)₈ (SEQ ID NO: 2) polypeptide). In some cases, an anti-polyGP polypeptide antibody (e.g., an anti-(GP)₈ (SEQ ID NO: 2) polypeptide antibody) provided herein can have little or no detectable binding to a (PGP)₈ (SEQ ID NO: 4) polypeptide or to a collagen polypeptide (e.g., a human collagen polypeptide). In some cases, the binding affinity of an anti-polyGP polypeptide antibody (e.g., an anti-(GP)₈ (SEQ ID NO: 2)

polypeptide antibody) provided herein for a polyGP polypeptide (e.g., a (GP)₈ (SEQ ID NO: 2) polypeptide) can be greater than 10³ mol⁻¹ (e.g., greater than 10³, 10⁴, 10⁵, 10⁶, 10⁷, 10⁸, 10⁹, 10¹⁰, 10¹¹, 10¹², or 10¹³ mol⁻¹). In some cases, the binding affinity of an anti-polyGP polypeptide antibody (e.g., an anti-(PG)₈ polypeptide antibody) provided
5 herein for a polyGP polypeptide (e.g., a (GP)₈ (SEQ ID NO: 2) polypeptide) can be between 10³ mol⁻¹ and 10¹³ mol⁻¹ (e.g., between 10³ mol⁻¹ and 10¹² mol⁻¹, between 10³ mol⁻¹ and 10¹¹ mol⁻¹, between 10³ mol⁻¹ and 10¹⁰ mol⁻¹, between 10³ mol⁻¹ and 10⁹ mol⁻¹, between 10³ mol⁻¹ and 10⁸ mol⁻¹, between 10³ mol⁻¹ and 10⁷ mol⁻¹, between 10⁴ mol⁻¹ and 10¹² mol⁻¹, between 10⁵ mol⁻¹ and 10¹² mol⁻¹, between 10⁶ mol⁻¹ and 10¹² mol⁻¹, between
10 10⁷ mol⁻¹ and 10¹² mol⁻¹, between 10⁸ mol⁻¹ and 10¹² mol⁻¹, between 10⁹ mol⁻¹ and 10¹² mol⁻¹, between 10¹⁰ mol⁻¹ and 10¹² mol⁻¹, or between 10⁶ mol⁻¹ and 10¹⁰ mol⁻¹). In some cases, the binding affinity of an anti-polyGP polypeptide antibody (e.g., an anti-(GP)₈ (SEQ ID NO: 2) polypeptide antibody) provided herein for a (PGP)₈ (SEQ ID NO: 4) polypeptide or to a collagen polypeptide (e.g., a human collagen polypeptide) can be less
15 than 10⁵ mol⁻¹ binding affinity (e.g., less than 10⁴, 10³, or 10² mol⁻¹).

Antibodies provided herein can be prepared using any appropriate method. For example, a polyGP polypeptide preparation or a substantially pure (GP)₈ (SEQ ID NO: 2) polypeptide (e.g., C-Ahx-GPGPGPGPGPGPGP-amide (SEQ ID NO: 1)) can be used as an immunogen to elicit an immune response in an animal such that specific antibodies
20 are produced. In some cases, tagged (e.g., GST-tagged) or untagged polypeptides having a (GP)₈₋₂₀₀₀ sequence can be used as an immunogen to elicit an immune response in an animal such that specific antibodies are produced. The immunogen used to immunize an animal can be chemically synthesized or derived from translated cDNA. For example, polypeptides having a (GP)₈₋₂₀₀₀ sequence can be expressed exogenously by cells (e.g.,
25 mammalian or bacteria cells) and isolated or used as a crude polypeptide extract. In some cases, the immunogen can be conjugated to a carrier polypeptide, if desired. Commonly used carriers that are chemically coupled to an immunizing polypeptide include, without limitation, m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid.

30 The preparation of polyclonal antibodies is well-known to those skilled in the art. See, e.g., Green *et al.*, Production of Polyclonal Antisera, in IMMUNOCHEMICAL

PROTOCOLS (Manson, ed.), pages 1 5 (Humana Press 1992) and Coligan et al.,
Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters, in CURRENT
PROTOCOLS IN IMMUNOLOGY, section 2.4.1 (1992). In addition, those of skill in
the art will know of various techniques common in the immunology arts for purification
5 and concentration of polyclonal antibodies, as well as monoclonal antibodies (Coligan, *et*
al., Unit 9, Current Protocols in Immunology, Wiley Interscience, 1994).

The preparation of monoclonal antibodies also is well-known to those skilled in
the art. See, e.g., Kohler & Milstein, *Nature* 256:495 (1975); Coligan *et al.*, sections
2.5.1 2.6.7; and Harlow *et al.*, ANTIBODIES: A LABORATORY MANUAL, page 726
10 (Cold Spring Harbor Pub. 1988). Briefly, monoclonal antibodies can be obtained by
injecting mice with a composition comprising an antigen, verifying the presence of
antibody production by analyzing a serum sample, removing the spleen to obtain B
lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas,
cloning the hybridomas, selecting positive clones that produce antibodies to the antigen,
15 and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be
isolated and purified from hybridoma cultures by a variety of well-established techniques.
Such isolation techniques include affinity chromatography with Protein A Sepharose, size
exclusion chromatography, and ion exchange chromatography. See, e.g., Coligan *et al.*,
sections 2.7.1 2.7.12 and sections 2.9.1 2.9.3; Barnes *et al.*, Purification of
20 Immunoglobulin G (IgG) , in METHODS IN MOLECULAR BIOLOGY, VOL. 10,
pages 79 104 (Humana Press 1992).

In addition, methods of *in vitro* and *in vivo* multiplication of monoclonal
antibodies are well known to those skilled in the art. Multiplication *in vitro* can be
carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or
25 RPMI 1640 medium, optionally replenished by mammalian serum such as fetal calf
serum, or trace elements and growth sustaining supplements such as normal mouse
peritoneal exudate cells, spleen cells, and bone marrow macrophages. Production *in vitro*
provides relatively pure antibody preparations and allows scale up to yield large amounts
of the desired antibodies. Large scale hybridoma cultivation can be carried out by
30 homogenous suspension culture in an airlift reactor, in a continuous stirrer reactor, or in
immobilized or entrapped cell culture. Multiplication *in vivo* may be carried out by

injecting cell clones into mammals histocompatible with the parent cells (e.g., osyngeneic mice) to cause growth of antibody producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

In some cases, the antibodies provided herein can be made using non-human primates. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in Goldenberg *et al.*, International Patent Publication WO 91/11465 (1991) and Losman *et al.*, *Int. J. Cancer*, 46:310 (1990).

In some cases, the antibodies can be humanized monoclonal antibodies. Humanized monoclonal antibodies can be produced by transferring mouse complementarity determining regions (CDRs) from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l. Acad. Sci. USA* 86:3833 (1989). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 321:522 (1986); Riechmann *et al.*, *Nature* 332:323 (1988); Verhoeyen *et al.*, *Science* 239:1534 (1988); Carter *et al.*, *Proc. Nat'l. Acad. Sci. USA* 89:4285 (1992); Sandhu, *Crit. Rev. Biotech.* 12:437 (1992); and Singer *et al.*, *J. Immunol.* 150:2844 (1993).

Antibodies provided herein can be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas *et al.*, *METHODS: A COMPANION TO METHODS IN ENZYMOLOGY*, VOL. 2, page 119 (1991) and Winter *et al.*, *Ann. Rev. Immunol.* 12: 433 (1994). Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In some cases, antibodies provided herein can be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions

of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens and can be used to produce human antibody secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.*, *Nature Genet.* 7:13 (1994); Lonberg *et al.*, *Nature* 368:856 (1994); and Taylor *et al.*, *Int. Immunol.* 6:579 (1994).

Antibody fragments can be prepared by proteolytic hydrolysis of an intact antibody or by the expression of a nucleic acid encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of intact antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. In some cases, an enzymatic cleavage using pepsin can be used to produce two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg (U.S. Patent Nos. 4,036,945 and 4,331,647). See also Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230 (1960); Porter, *Biochem. J.* 73:119 (1959); Edelman *et al.*, *METHODS IN ENZYMOLOGY*, VOL. 1, page 422 (Academic Press 1967); and Coligan *et al.* at sections 2.8.1 2.8.10 and 2.10.1 2.10.4.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used provided the fragments retain some ability to bind (e.g., selectively bind) its epitope.

The antibodies provided herein can be substantially pure. The term "substantially pure" as used herein with reference to an antibody means the antibody is substantially free of other polypeptides, lipids, carbohydrates, and nucleic acid with which it is naturally associated. Thus, a substantially pure antibody is any antibody that is removed from its natural environment and is at least 60 percent pure. A substantially pure antibody can be at least about 65, 70, 75, 80, 85, 90, 95, or 99 percent pure.

In some cases, biological samples obtained from C9⁺ FTLD and C9⁺ ALS patients can contain detectable levels or elevated levels of polyGA polypeptides, polyGR

polypeptides, polyPA polypeptides, and/or polyPR polypeptides in addition to polyGP polypeptides generated from the expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of C9ORF72 present within the C9⁺ FTLD or C9⁺ ALS patient. In such cases, antibodies generated against polyGA polypeptides (e.g., antibodies generated against C-Ahx-GAGAGAGAGAGAGAGA-amide (SEQ ID NO: 6)), or a GST-tagged (GA)₅₀ polypeptide that was exogenously expressed in bacteria and isolated), antibodies generated against polyGR polypeptides (e.g., antibodies generated against C-Ahx-GRGRGRGRGRGRGR-amide (SEQ ID NO: 7)), or a GST-tagged (GR)₅₀ polypeptide that was exogenously expressed in bacteria and isolated), antibodies generated against polyPA polypeptides (e.g., antibodies generated against C-Ahx-PAPAPAPAPAPAPAPA-amide (SEQ ID NO: 12)), or a GST-tagged (PA)₅₀ polypeptide that was exogenously expressed in bacteria and isolated), and/or antibodies generated against polyPR polypeptides (e.g., antibodies generated against C-Ahx-PRPRPRPRPRPRPR-amide (SEQ ID NO: 13)) or a GST-tagged (PR)₅₀ polypeptide that was exogenously expressed in bacteria and isolated) can be used instead of or in combination with antibodies generated against polyGP polypeptides (e.g., antibodies generated against C-Ahx-GPGPGPGPGPGPGP-amide; SEQ ID NO: 1) as described herein to detect those detectable or elevated levels of polyGA polypeptides, polyGR polypeptides, polyPA polypeptides, polyPR polypeptides, and/or polyGP polypeptides within biological samples, thereby identifying the patient as having C9⁺ FTLD or C9⁺ ALS. In addition, the anti-polyGA polypeptide antibodies (e.g., antibodies generated against C-Ahx-GAGAGAGAGAGAGAGA-amide (SEQ ID NO: 6)), anti-polyGR polypeptide antibodies (e.g., antibodies generated against C-Ahx-GRGRGRGRGRGRGR-amide (SEQ ID NO: 7)), anti-polyPA polypeptide antibodies (e.g., antibodies generated against C-Ahx-PAPAPAPAPAPAPAPA-amide (SEQ ID NO: 12)), and anti-polyPR polypeptide antibodies (e.g., antibodies generated against C-Ahx-PRPRPRPRPRPRPR-amide (SEQ ID NO: 13)) provided herein can be made and used using methods similar to those described herein with respect to the anti-polyGP polypeptide antibodies.

The invention will be further described in the following example, which does not limit the scope of the invention described in the claims.

EXAMPLE

Example 1 – Producing a preparation containing anti-polyGP polypeptide antibodies

The expansion of a hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a
5 noncoding region of C9ORF72 was hypothesized to form an mRNA sense transcript with
the capability of expressing polyGA polypeptides, polyGP polypeptides, and polyGR
polypeptides (Figure 1). To test this hypothesis, a polyclonal antibody preparation was
produced as follows.

Briefly, two rabbits were immunized by injection with a mixture of the following
10 polypeptides: C-Ahx-GAGAGAGAGAGAGAGA-amide (SEQ ID NO: 6), C-Ahx-
GPGPGPGPGPGPGP-amide (SEQ ID NO: 1), and C-Ahx-
GRGRGRGRGRGRGR-amide (SEQ ID NO: 7). Prior to immunization of the
rabbits, the purity of each polypeptide was verified by mass spectrometry, and each
polypeptide was conjugated to m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS)
15 as an immune carrier. Repeat immunizations were performed, and bleeds were
subsequently collected to obtain antiserum.

Antiserum was directly used for some experimental investigations, such as
immunohistochemical analysis of C9ORF72 translated polypeptides in human brain
sections (Figure 3 and Figure 5). In addition, polyclonal antibodies were purified from
20 the antiserum with Protein A or Protein G. This generated a pool of antibodies that
preferentially detect polyGP polypeptides (Figure 2). The polyclonal antibody
preparation produced was designated the MC-001 antibody.

The MC-001 antibody bound to (GP)₈ (SEQ ID NO: 2) polypeptides, and not
(GA)₈ (SEQ ID NO: 8) polypeptides, (GR)₈ (SEQ ID NO: 9) polypeptides, (GK)₈ (SEQ
25 ID NO: 10) polypeptides, and (GX)₈ (SEQ ID NO: 11) polypeptides (Figures 2A and 2B).

Example 2 – Anti-polyGP polypeptide antibodies are capable of detecting C9⁺ FLTD and C9⁺ ALS patients

The MC-001 antibody was capable of specific detection of insoluble inclusions of
30 polyGP polypeptides in the brains of patients with the C9ORF72 GGGGCC (SEQ ID
NO: 3) genetic expansion. Cerebellar, hippocampal, and frontocortical sections exhibited

specific MC-001 immunoreactivity by immunohistochemistry in FTLD patients with the genetic GGGGCC (SEQ ID NO: 3) expansion (Figure 3), but not in sections from FTLD patients without the genetic expansion. No MC-001 immunoreactivity was detected in brain tissue sections from affected areas of Alzheimer's disease (AD) or the trinucleotide repeat disorders spinocerebellar ataxia type 3 (SCA3) or Huntington's disease (HD) (Figure 4). These results indicate that MC-001 antibody immunoreactivity can be highly specific only to patients with the C9ORF72 GGGGCC (SEQ ID NO: 3) genetic expansion.

Sequential extraction of protein from the cerebellum of C9⁺FTD or C9⁺ALS cases with increasingly stringent detergents revealed that the MC-001 antibody labeled insoluble high molecular weight material. Immunoblots of the 7M urea fractions from cerebellar tissue revealed the presence of MC-001 immunoreactive material that, due to its large size and insolubility, became trapped in the top of the SDS-PAGE stacking gel (Figure 5A). To assess MC-001 immunoreactivity, dot-blot (Figure 5B) and MSD immunoassay (Figure 5C) were conducted to overcome the problem of the immobility of the material by SDS-PAGE. The presence of high molecular weight insoluble material in C9⁺ FTD/ALS brain was comparable to other neurodegenerative disorders with insoluble neuronal inclusions such as insoluble tau inclusions in Alzheimer's disease. The MC-001 antibody only exhibited immunoreactivity to insoluble material extracted from brain regions from patients with the C9ORF72 GGGGCC (SEQ ID NO: 3) genetic expansion.

Example 3 – Anti-polyGP polypeptide antibodies are capable of detecting C9⁺ ALS patients using cerebrospinal fluid

To evaluate the potential of the MC-001 antibody further, cerebrospinal fluid from two normal controls, six ALS patients negative for C9ORF72 expanded repeats, and one C9⁺ ALS patient was assessed using the MSD platform. As shown in Figure 6, MC-001 immunoreactivity in the C9⁺ALS CSF was increased more than threefold the standard error of the mean signal in CSF from control ALS patients (indicated by dashed line). These results demonstrate that the presence of polypeptides such as polyGP polypeptides in patient cerebrospinal fluid can serve as a detectable marker for both diagnostic and prognostic tests for C9⁺ FTD and ALS.

Example 4 – Producing goat anti-polyGP antibodies

One goat was immunized by injection with a C-Ahx-GPGPGPGPGPGPGP-
amide (SEQ ID NO: 1) polypeptide. Prior to immunization, the purity of the polypeptide
5 was verified by mass spectrometry, and the polypeptide was conjugated to m-
maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as an immune carrier. Repeat
immunizations were performed, and bleeds were subsequently collected to obtain
antiserum. Subsequent antibody purification by Protein A or Protein G, or antigen-
specific purification, was performed.

10

Example 5 – Producing rabbit anti-polyGP antibodies

Two rabbits were immunized by injection with a C-Ahx-
GPGPGPGPGPGPGP-amide (SEQ ID NO: 1) polypeptide. Prior to immunization,
the purity of the polypeptide was verified by mass spectrometry, and the polypeptide was
15 conjugated to m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as an immune
carrier. Repeat immunizations are performed, and bleeds are subsequently collected to
obtain antiserum. Subsequent antibody purification by Protein A or Protein G, or antigen-
specific purification, is performed.

20

Example 6 – Producing rabbit anti-polyGA antibodies

Two rabbits were immunized by injection with a C-Ahx-
GAGAGAGAGAGAGAGA-amide (SEQ ID NO: 6) polypeptide. Prior to immunization,
the purity of the polypeptide was verified by mass spectrometry, and the polypeptide was
conjugated to m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as an immune
25 carrier. Repeat immunizations were performed, and bleeds were subsequently collected
to obtain antiserum. Subsequent antibody purification by Protein A or Protein G, or
antigen-specific purification, was performed.

30

Two rabbits were immunized by injection with recombinant (GA)₅₀ tagged with
glutathione S-transferase (GST). In brief, synthetic cDNA encoding (GA)₅₀ was inserted
into a pGEX-6P-1 vector (GE Healthcare) downstream of the GST sequence, and the
plasmid was transfected into Rosetta™(DE3)pLysS Competent Cells

(EMD4Biosciences). For induction of recombinant GST-(GA)₅₀ polypeptide, bacteria were cultured overnight in the presence of isopropyl β-D-1-thiogalactopyranoside. Cells were then lysed, sonicated, and centrifuged at 18000 x g for 30 minutes. The resulting supernatant was applied to a Glutathione Sepharose 4B column, and the recombinant GST-(GA)₅₀ polypeptide was eluted from the column using Tris-Cl (50 mM, pH 8.0) containing 20 mM reduced glutathione. After removal of glutathione from the GST-(GA)₅₀ preparation, GST-(GA)₅₀ was used for immunization of rabbits.

Repeat immunizations are performed, and bleeds are subsequently collected to obtain antiserum. Subsequent antibody purification by Protein A or Protein G, or antigen-specific purification, is performed.

Example 7 – Producing rabbit anti-polyGR antibodies

Two rabbits were immunized by injection with a C-Ahx-GRGRGRGRGRGRGRGR-amide (SEQ ID NO: 7) polypeptide. Prior to immunization, the purity of the polypeptide was verified by mass spectrometry, and the polypeptide was conjugated to m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as an immune carrier. Repeat immunizations are performed, and bleeds are subsequently collected to obtain antiserum. Subsequent antibody purification by Protein A or Protein G, or antigen-specific purification, is performed.

Example 8 – Producing mouse monoclonal anti-polyGP antibodies

Five mice were immunized by injection with a mixture of C-Ahx-GAGAGAGAGAGAGAGA-amide (SEQ ID NO: 6), C-Ahx-GPGPGPGPGPGPGP-amide (SEQ ID NO: 1), and C-Ahx-GRGRGRGRGRGRGRGR-amide (SEQ ID NO: 7) polypeptides. Prior to immunization, the purity of the polypeptide was verified by mass spectrometry, and the polypeptide was conjugated to m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as an immune carrier.

B cells were isolated from the spleen of immunized mice and were fused with myeloma cells to create hybridomas. Hybridomas were cultured under conditions which permit growth of only hybridoma cells where each cell produces an antibody against a single epitope. Single hybridoma cells were separated and were grown in distinct wells

of a microtiter plate. Each hybridoma was screened to determine whether it produces the desired antibody (i.e., antibody against (GP)₈ (SEQ ID NO: 2), (GA)₈ (SEQ ID NO: 8), or (GR)₈ (SEQ ID NO: 9)). The hybridoma cells producing a desired monoclonal antibody are cultured, and monoclonal antibodies are harvested from the supernatant. Monoclonal antibody purification from the supernatant is performed by affinity purification using Protein A or Protein G, or using immobilized antigen (i.e., (GP)₈ (SEQ ID NO: 2), (GA)₈ (SEQ ID NO: 8), or (GR)₈ (SEQ ID NO: 9) polypeptides).

Antibody clones were tested for the ability to bind (PA)₈, (PR)₈, (GP)₈, (GA)₈, or (GR)₈ polypeptides. Briefly, (PA)₈, (PR)₈, (GP)₈, (GA)₈, and (GR)₈ polypeptides were adsorbed individually to carbon electrodes at the bottom of 96-well MSD™ assay plates. After immobilization and blocking, antibodies from individual clones in combination with a Sulfo-tagged rabbit secondary antibody were added to the wells. Immunoreactivity was measured by adding MSD™ 1x Read Buffer to the wells and reading the light emission at 620 nm after electrochemical stimulation using the MSD™ Sector Imager 2400.

Three monoclonal antibody clones (clones G2, G3, and H4) produced monoclonal antibodies that bound to the (GP)₈ polypeptide with no detectable binding to the (PA)₈, (PR)₈, (GA)₈, and (GR)₈ polypeptides (Figure 7).

Example 9 – Producing rabbit anti-polyPA antibodies

Two rabbits were immunized by injection with the following polypeptide antigen: C-Ahx-PAPAPAPAPAPAPAPA-amide (SEQ ID NO: 12).

Prior to immunization, the polypeptide purity was verified by mass spectrometry, and the polypeptide was conjugated to m-Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as an immune carrier. Repeat immunizations are performed, and bleeds are collected to obtain antiserum. Subsequent antibody purification by Protein A or Protein G, or antigen-specific purification, is performed.

Example 10 – Producing rabbit anti-polyPR antibodies

Two rabbits were immunized by injection with the following polypeptide antigen: C-Ahx-PRPRPRPRPRPRPR-amide (SEQ ID NO: 13).

Prior to immunization, the polypeptide purity was verified by mass spectrometry, and the polypeptide was conjugated to m-Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as an immune carrier. Repeat immunizations are performed, and bleeds are collected to obtain antiserum. Subsequent antibody purification by Protein A or Protein
5 G, or antigen-specific purification, is performed.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction
with the detailed description thereof, the foregoing description is intended to illustrate
10 and not limit the scope of the invention, which is defined by the scope of the appended
claims. Other aspects, advantages, and modifications are within the scope of the
following claims.

WHAT IS CLAIMED IS:

1. A method for identifying a mammal having an expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat in a noncoding region of at least one C9ORF72 allele, wherein said method comprises:

- 5 (a) contacting a biological sample obtained from said mammal with an anti-polyGP antibody under conditions wherein a polyGP polypeptide present within said biological sample and said anti-polyGP antibody form a polyGP polypeptide/anti-polyGP antibody complex,
- (b) detecting said polyGP polypeptide/anti-polyGP antibody complex, and
- 10 (c) classifying said mammal as having said expanded hexanucleotide (GGGGCC) (SEQ ID NO: 3) repeat.

2. The method of claim 1, wherein said mammal is a human.

15 3. The method of any one of claims 1-2, wherein said biological sample is a cerebrospinal fluid sample.

4. The method of any one of claims 1-2, wherein said biological sample is a blood sample.

20

5. The method of any one of claims 1-4, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide is between 10^6 mol^{-1} and 10^{12} mol^{-1} .

25

6. The method of any one of claims 1-4, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide is between 10^6 mol^{-1} and 10^{12} mol^{-1} , and wherein the binding affinity of said anti-polyGP antibody for a (PGP)₈ (SEQ ID NO: 4) polypeptide is less than 10^3 mol^{-1} .

7. A method for identifying a human as having C9⁺ neurological condition, wherein said method comprises:

(a) contacting a biological sample obtained from a human with an anti-polyGP antibody under conditions wherein a polyGP polypeptide present within said biological sample and said anti-polyGP antibody form a polyGP polypeptide/anti-polyGP antibody complex,

(b) detecting said polyGP polypeptide/anti-polyGP antibody complex, and

(c) classifying said human as having a C9⁺ neurological condition.

8. The method of claim 7, wherein said biological sample is a cerebrospinal fluid sample.

9. The method of any one of claims 7-8, wherein said biological sample is a blood sample.

10. The method of any one of claims 7-9, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide is between 10⁶ mol⁻¹ and 10¹² mol⁻¹.

11. The method of any one of claims 7-9, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ polypeptide is between 10⁶ mol⁻¹ and 10¹² mol⁻¹, and wherein the binding affinity of said anti-polyGP antibody for a (PGP)₈ (SEQ ID NO: 4) polypeptide is less than 10³ mol⁻¹.

12. The method of any one of claims 7-11, wherein said C9⁺ neurological condition is C9⁺ FTLD.

13. The method of any one of claims 7-11, wherein said C9⁺ neurological condition is C9⁺ ALS.

14. An anti-polyGP antibody.

15. The anti-polyGP antibody of claim 14, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide is between 10⁶ mol⁻¹ and 10¹² mol⁻¹.

5

16. The anti-polyGP antibody of any one of claims 14-15, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide is between 10⁶ mol⁻¹ and 10¹² mol⁻¹, and wherein the binding affinity of said anti-polyGP antibody for a (PGP)₈ (SEQ ID NO: 4) polypeptide is less than 10³ mol⁻¹.

10

17. The anti-polyGP antibody of any one of claims 14-16, wherein said anti-polyGP antibody is an anti-(GP)₈ (SEQ ID NO: 2) antibody.

18. An antibody preparation comprising an anti-polyGP antibody.

15

19. The antibody preparation of claim 18, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide is between 10⁶ mol⁻¹ and 10¹² mol⁻¹.

20

20. The antibody preparation of any one of claims 18-19, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ (SEQ ID NO: 2) polypeptide is between 10⁶ mol⁻¹ and 10¹² mol⁻¹, and wherein the binding affinity of said anti-polyGP antibody for a (PGP)₈ (SEQ ID NO: 4) polypeptide is less than 10³ mol⁻¹.

25

21. The antibody preparation of any one of claims 18-20, wherein said anti-polyGP antibody is an anti-(GP)₈ (SEQ ID NO: 2) antibody.

22. A method for detecting a polyGP polypeptide present within said sample, wherein said method comprises:

(a) contacting said biological sample with an anti-polyGP antibody under conditions wherein said polyGP polypeptide present within said sample and said anti-polyGP antibody form a polyGP polypeptide/anti-polyGP antibody complex, and

(b) detecting said polyGP polypeptide/anti-polyGP antibody complex, thereby
5 detecting the presence of said polyGP polypeptide within said sample.

23. The method of claim 22, wherein said biological sample is a cerebrospinal fluid sample.

10 24. The method of claim 22, wherein said biological sample is a blood sample.

25. The method of any one of claims 22-24, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ polypeptide is between 10^6 mol^{-1} and 10^{12} mol^{-1} .

15 26. The method of any one of claims 22-24, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ polypeptide is between 10^6 mol^{-1} and 10^{12} mol^{-1} , and wherein the binding affinity of said anti-polyGP antibody for a (PGP)₈ polypeptide is less than 10^3 mol^{-1} .

20 27. The method of any one of claims 22-26, wherein said anti-polyGP antibody is a monoclonal antibody.

28. A method for detecting the presence or absence of a polyGP polypeptide within said sample, wherein said method comprises:

25 (a) contacting said biological sample with an anti-polyGP antibody under conditions wherein said polyGP polypeptide, if present within said sample, and said anti-polyGP antibody form a polyGP polypeptide/anti-polyGP antibody complex, and

(b) determining the presence or absence of said polyGP polypeptide/anti-polyGP antibody complex from step (a), wherein the presence of said complex indicates the
30 presence of said polyGP polypeptide within said sample, and wherein the absence of said complex indicates the absence of said polyGP polypeptide within said sample.

29. The method of claim 28, wherein said biological sample is a cerebrospinal fluid sample.
- 5 30. The method of claim 28, wherein said biological sample is a blood sample.
31. The method of any one of claims 28-30, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ polypeptide is between 10^6 mol^{-1} and 10^{12} mol^{-1} .
- 10 32. The method of any one of claims 28-30, wherein the binding affinity of said anti-polyGP antibody for a (GP)₈ polypeptide is between 10^6 mol^{-1} and 10^{12} mol^{-1} , and wherein the binding affinity of said anti-polyGP antibody for a (PGP)₈ polypeptide is less than 10^3 mol^{-1} .
- 15 33. The method of any one of claims 28-32, wherein said anti-polyGP antibody is a monoclonal antibody.

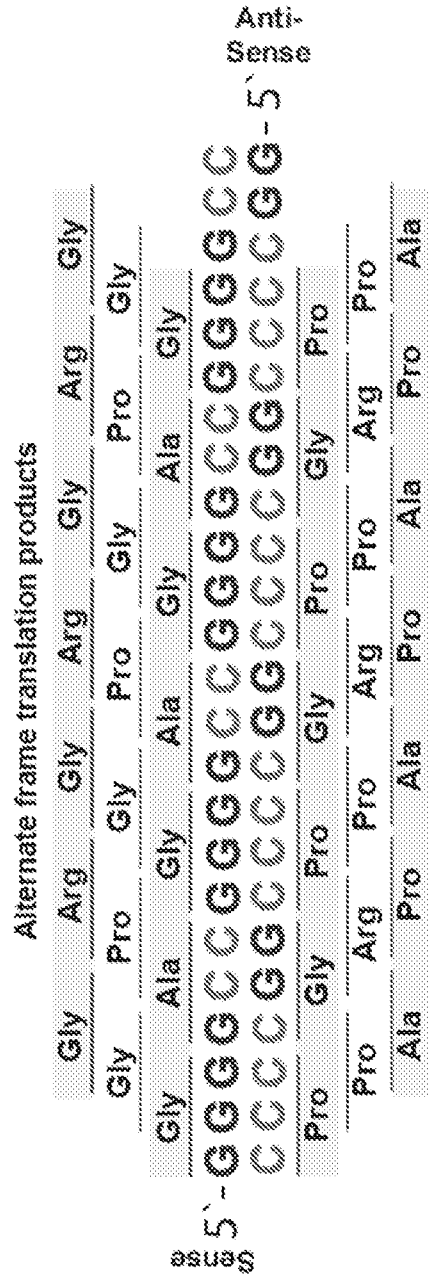


Figure 1

Figure 2

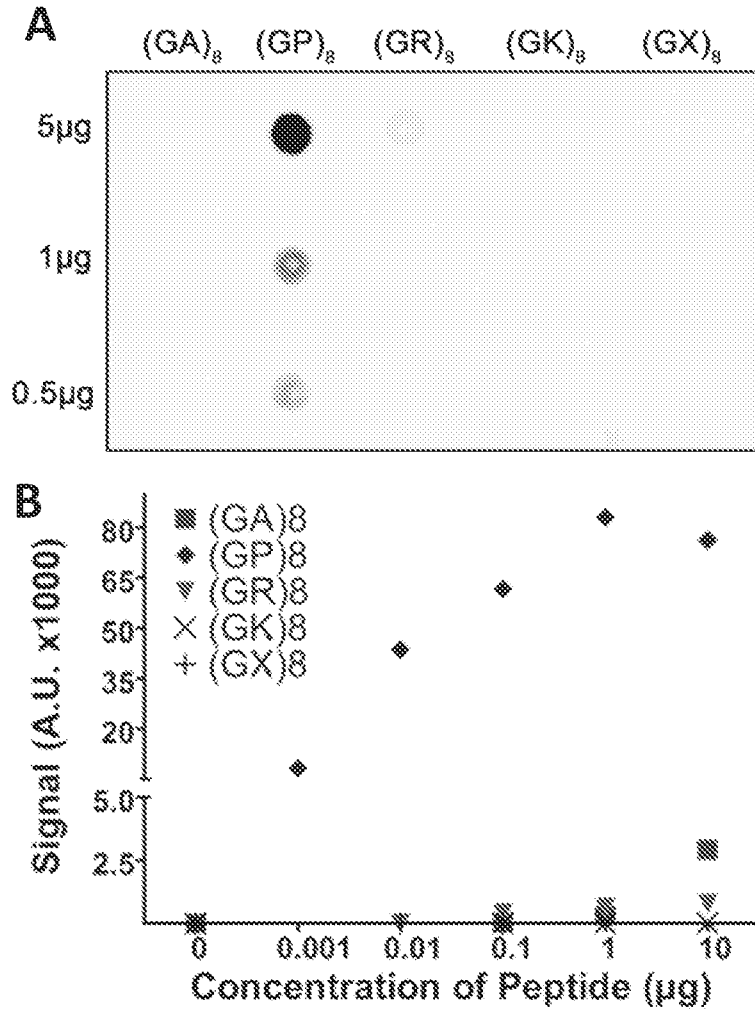


Figure 3

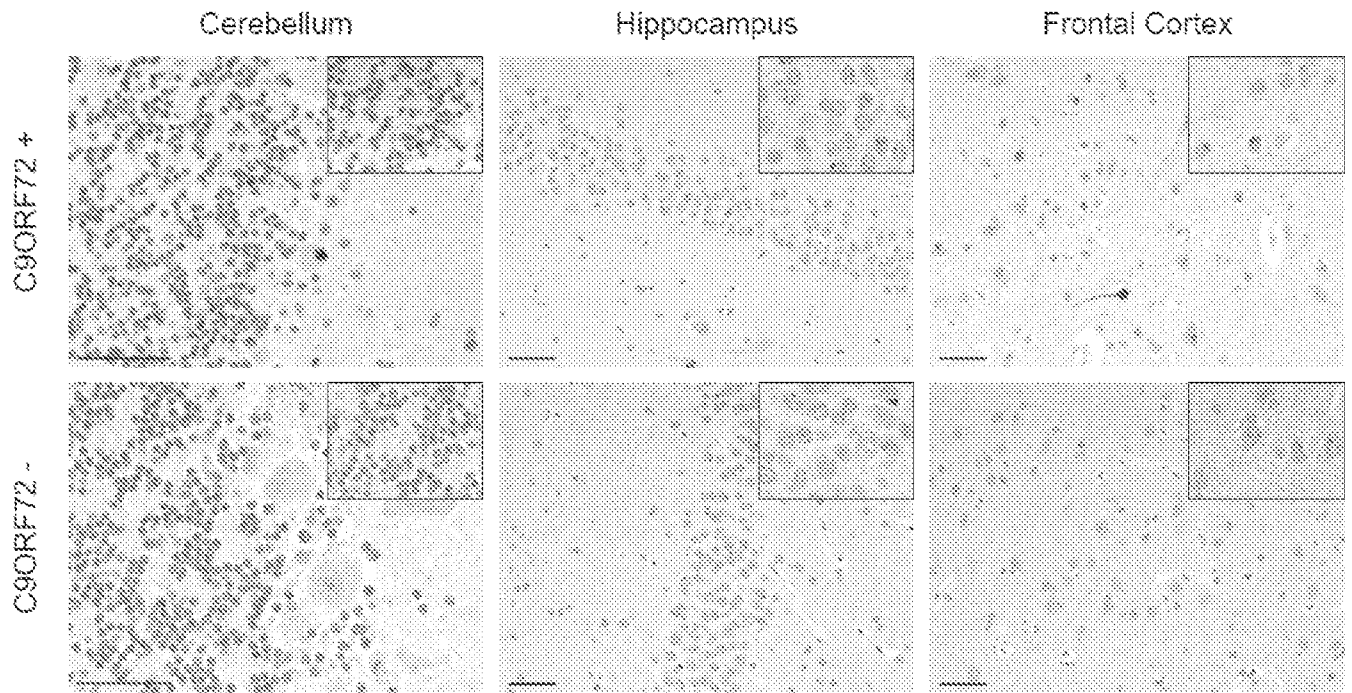


Figure 4

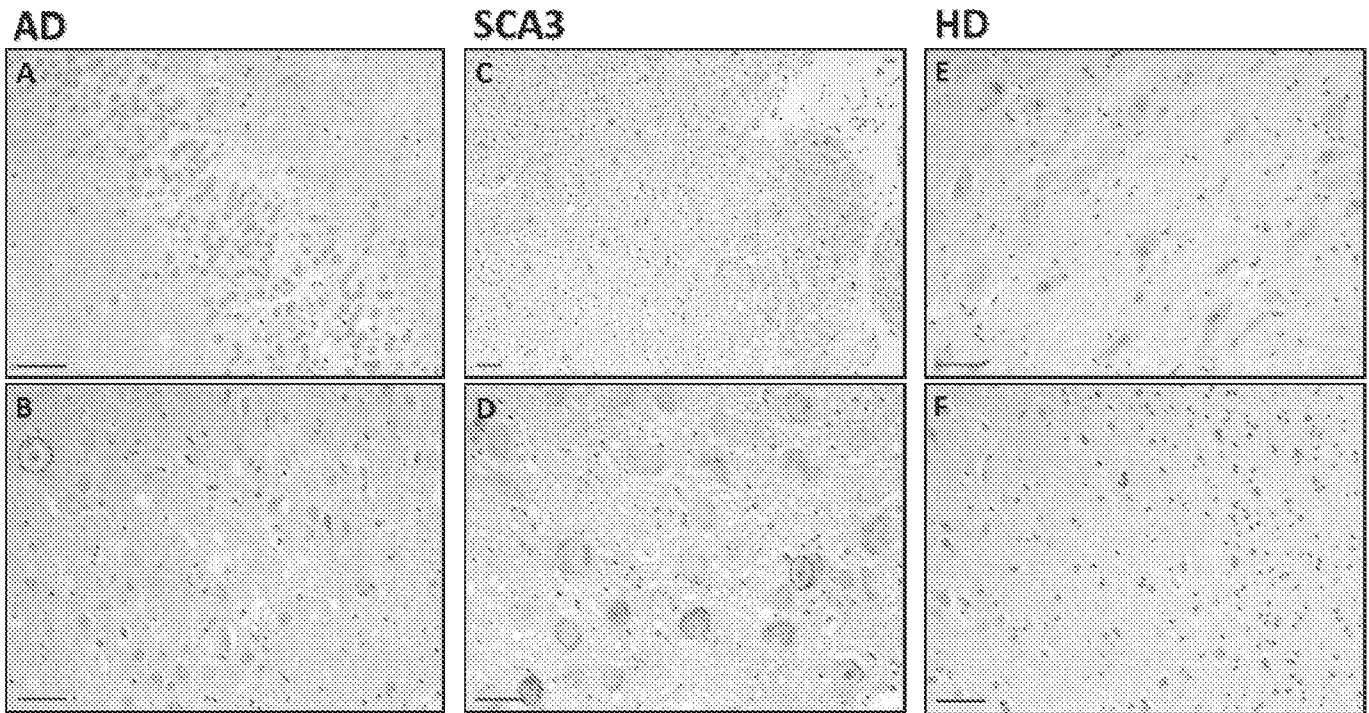


Figure 5

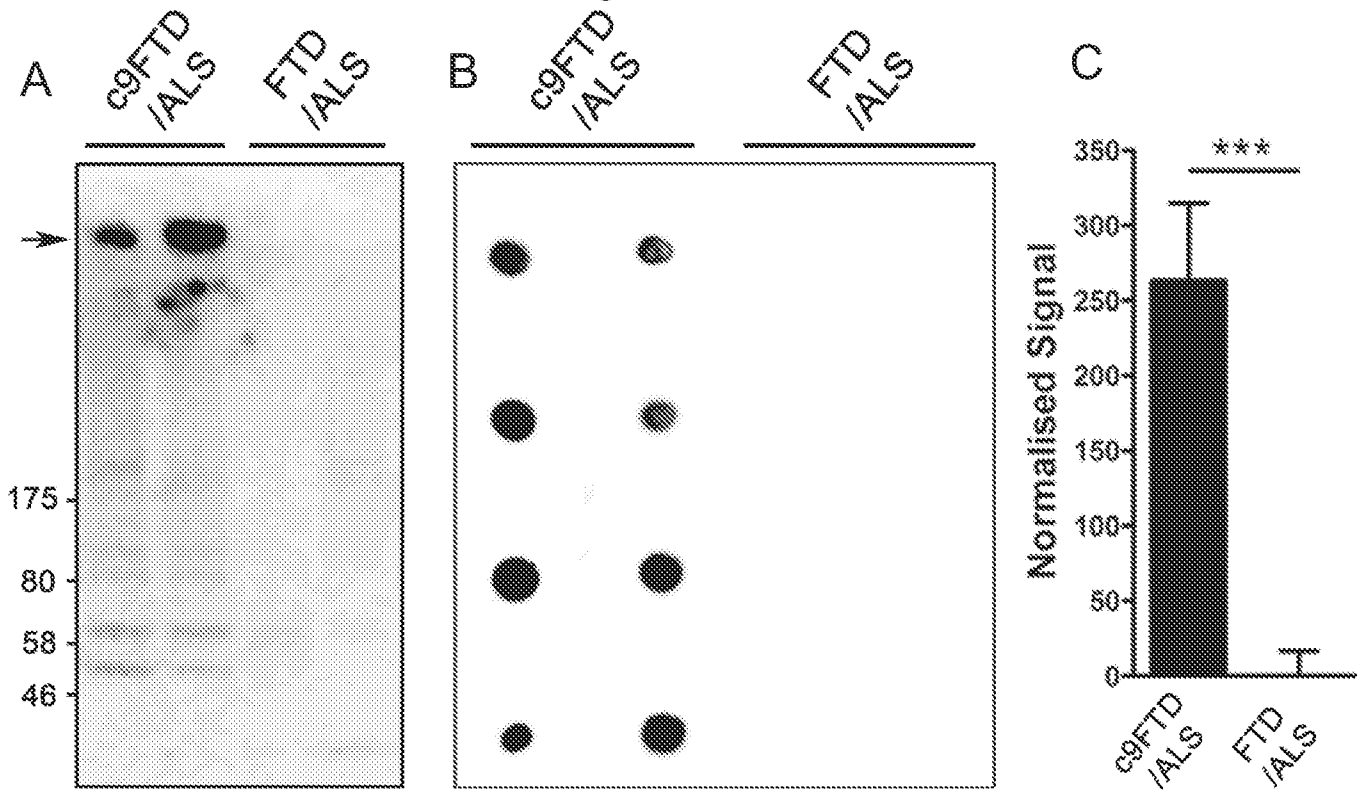


Figure 6

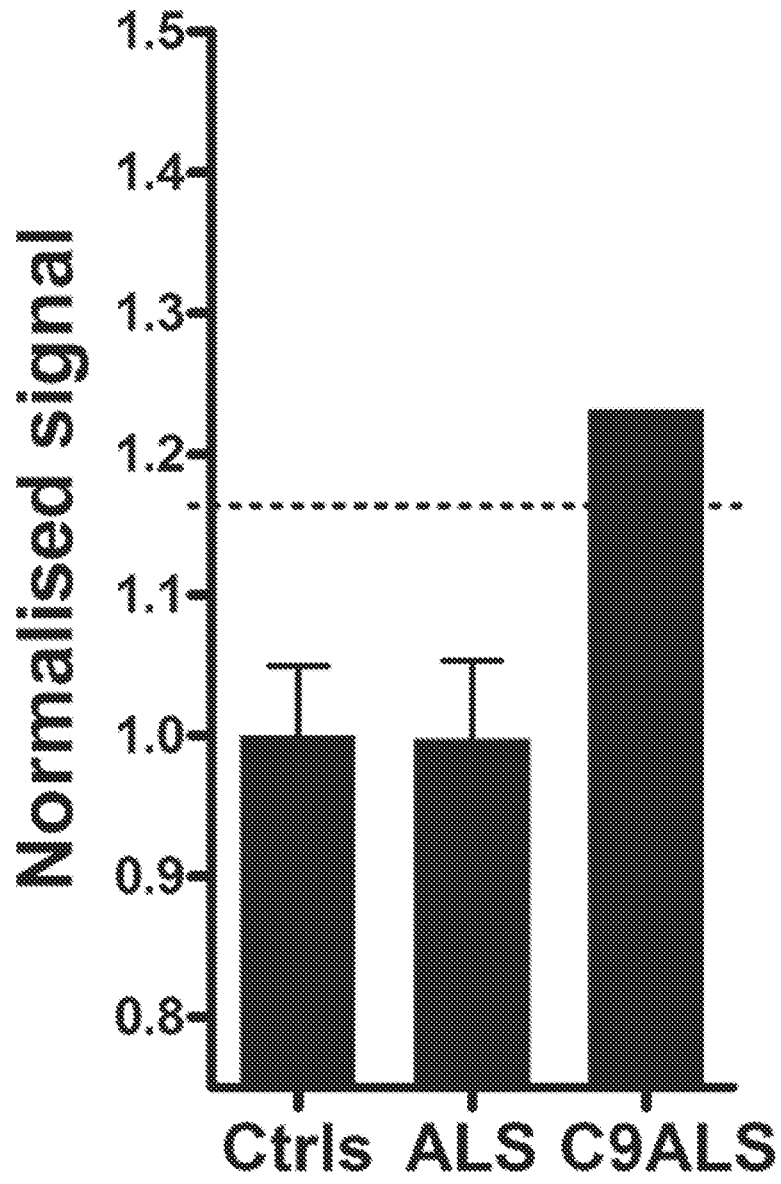
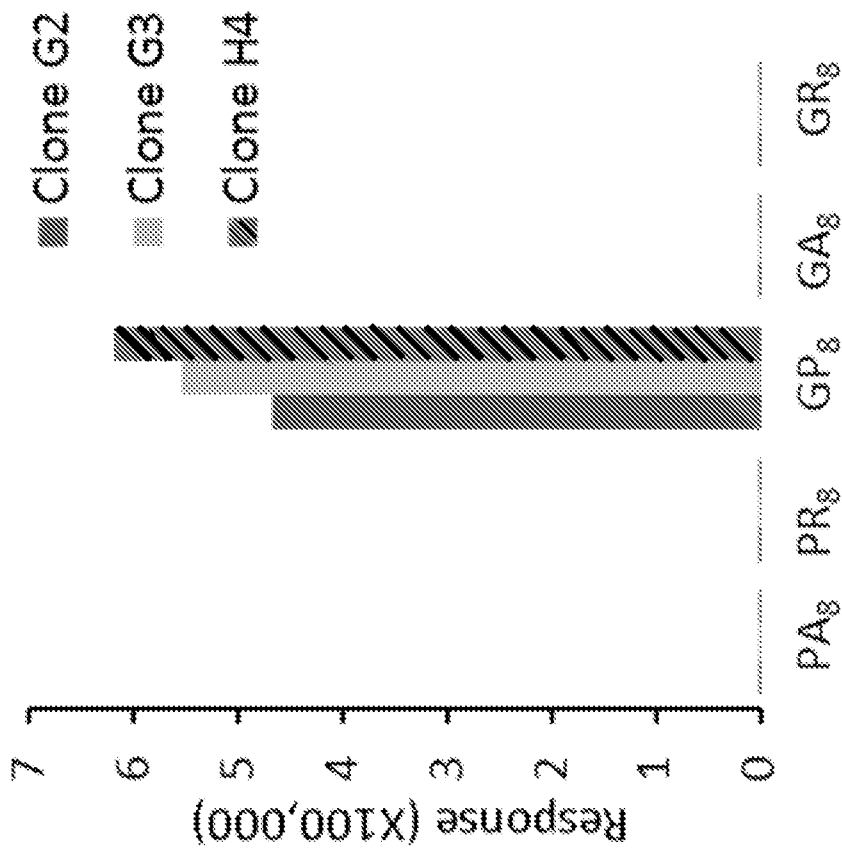


Figure 7



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2014/012801

A. CLASSIFICATION OF SUBJECT MATTER		<p style="text-align: center;"><i>C07K 16/18 (2006.01)</i> <i>G01N 33/53 (2006.01)</i></p>	
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)			
C07K 16/18, G01N 33/53			
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)			
PatSearch (RUPTO internal), EMBL, NCBI, PAJ, Espacenet, DWPI, USPTO DB, CIPO (Canada PO), SIPO DB			
C. DOCUMENTS CONSIDERED TO BE RELEVANT			
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
A	MARIELY DE JESUS-HERNANDEZ et al. Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS, Neuron, 2011, Vol. 72, p. 1-12	1-33	
A	US 2011/0171687 A1 (AMUNIX OPERATING INC et al) 14.07.2011, abstract, paragraphs [0102], [0119], SEQ ID NO: 66.	1-33	
A	BOEVE F. B. et al. Characterization of frontotemporal dementia and/or amyotrophic lateral sclerosis associated with the GGGGCC repeat expansion in C9ORF72, Brain, 2012, v.135, pp. 765-783, especially abstract, pp. 766-781.	1-33	
<input type="checkbox"/> Further documents are listed in the continuation of Box C.		<input type="checkbox"/> See patent family annex.	
* Special categories of cited documents:		<p>“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</p> <p>“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</p> <p>“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</p> <p>“&” document member of the same patent family</p>	
“A” document defining the general state of the art which is not considered to be of particular relevance	“E” earlier document but published on or after the international filing date		
“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	“O” document referring to an oral disclosure, use, exhibition or other means		
“P” document published prior to the international filing date but later than the priority date claimed			
Date of the actual completion of the international search	Date of mailing of the international search report		
21 April 2014 (21.04.2014)	29 May 2014 (29.05.2014)		
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1	Authorized officer		
Facsimile No. +7 (499) 243-33-37	K.Paramonova		
	Telephone No. 495 531 65 15		

专利名称(译)	检测c9ORF72六核苷酸重复扩增阳性额颞叶变性或c9ORF72六核苷酸重复扩增阳性肌萎缩侧索硬化的方法和材料		
公开(公告)号	EP2948471A1	公开(公告)日	2015-12-02
申请号	EP2014743115	申请日	2014-01-23
[标]申请(专利权)人(译)	梅约医学教育与研究基金会		
申请(专利权)人(译)	梅奥基金会的医学教育和研究		
当前申请(专利权)人(译)	梅奥基金会的医学教育和研究		
[标]发明人	ASH PETER E PETRUCELLI LEONARD GENDRON TANIA		
发明人	ASH, PETER, E. PETRUCELLI, LEONARD GENDRON, TANIA		
IPC分类号	C07K16/18 G01N33/53		
CPC分类号	G01N33/6896 C07K16/44 G01N33/5308 G01N2800/28		
代理机构(译)	Grund的 , MARTIN		
优先权	61/756318 2013-01-24 US		
其他公开文献	EP2948471A4		
外部链接	Espacenet		

摘要(译)

该文献提供了用于检测C9ORF72六核苷酸 (GGGGCC) (SEQ ID NO : 3) 重复扩增阳性 (C9 +) 额颞叶变性或C9 +肌萎缩侧索硬化的方法和材料。例如, 提供了与使用抗 (GP) 8 (SEQ ID NO : 2) 抗体鉴定具有C9 + FTLD或C9 + ALS的哺乳动物 (例如人) 相关的方法和材料。