

they need, nor do they or their care-givers qualify for critical support programs. Early diagnosis and early intervention are two important means of narrowing the treatment gap. Thus, early diagnostic tools that can determine disease risk both quickly and accurately have significant therapeutic value on many levels. Research has confirmed that AD affects the brain long before actual symptoms of memory loss or cognitive decline actually manifest. To this date, however, there are few diagnostic tools for early detection, see, *e.g.*, WO2021/228125. In most cases, by the time a patient is diagnosed with AD using methods currently in practice, which involves subjective clinical assessment, the pathological symptoms are typically already at an advanced state. As such, for the purpose of improving AD treatment and long-term management, there exists an urgent need for developing more new and effective methods for early diagnosis of AD in a patient or for detecting an increased risk of a patient developing AD at a future time. This invention addresses this and other related needs by disclosing novel methods and kits related to the use of circulating protein Nell-1 as a biomarker for assessing individual risk of developing neurodegenerative disorders such as the Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), and Parkinson's Disease (PD) and as a therapeutic target for treating neurodegenerative disorders.

[0005] BRIEF SUMMARY

[0006] The disclosure relates to the discovery of novel plasma protein markers associated with a neurodegenerative disorder such as the Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), and Parkinson's Disease (PD). The disclosure thus provides methods and compositions useful for diagnosis of these neurodegenerative disorders as well as for indicating therapeutic efficacy of an agent for treating the neurodegenerative disorders. As such, in a first aspect, the present disclosure provides a method for diagnosing, monitoring, or assessing risk for a neurodegenerative disorder in a subject, comprising: (1) comparing the subject's plasma or serum or whole blood level of Nell-1 protein with a standard control level of Nell-1 protein found in the plasma or serum or whole blood of an average healthy subject not suffering from or at increased risk for the neurodegenerative disorder; and (2) detecting a decrease in the subject's plasma or serum or whole blood level of Nell-1 protein from the standard control level, and determining the subject as suffering from or having an increased risk for the neurodegenerative disorder; or (3) detecting no decrease in the subject's plasma or serum or whole blood level of Nell-1 protein from the standard control level, and determining the subject as not suffering from or having no increased risk for the neurodegenerative disorder. In a first example, the present disclosure provides a method for assessing a subject's risk of developing a neurodegenerative disorder such as AD, MCI, or PD at a future time. The method includes the following steps: first, comparing the subject's plasma or serum or whole blood level of Nell-1 protein with a standard control level of Nell-1 protein found in the plasma or serum or whole blood of an average healthy subject not suffering from or at increased risk for the neurodegenerative disorder. Second, either detecting a decrease in the subject's plasma or serum or whole blood level of Nell-1 protein from the standard control level, and determining the subject as suffering from or having an increased risk for the neurodegenerative disorder; or, as an alternative to the second step, a third step of detecting no decrease in the subject's plasma or serum or whole blood level of Nell-1 protein from the standard control level, and determining the subject as not suffering from or having no increased risk for the neurodegenerative disorder. In some examples, this method further comprises, prior to the first step, a step of measuring the plasma or serum or whole blood level of Nell-1 protein. In some

examples, the method further comprises, prior to the above-mentioned measuring step, a step of obtaining a plasma or serum or whole blood sample from the subject. In some examples, the method further comprises, after the third step, a step of comparing the subject's plasma or serum or whole blood level of Nell-1 protein as measured at a later time (*i.e.*, at a second time point) with the subject's plasma or serum or whole blood level of Nell-1 protein in the first step (*i.e.*, at the original time point, or an earlier or first time point), wherein a higher plasma or serum or whole blood level of Nell-1 protein as measured at the later time indicates improvement of the neurodegenerative disorder, and wherein a lower plasma or serum or whole blood level of Nell-1 protein as measured at the later time indicates deterioration of the neurodegenerative disorder. In some examples, between the third step (or the first time) and the later time (or the second time), the subject has been administered a therapeutic agent intended for treating the neurodegenerative disorder. In some examples, the neurodegenerative disorder for which risk is being assessed is Alzheimer's Disease (AD). In some examples, the neurodegenerative disorder being assessed is Mild Cognitive Impairment (MCI) or Parkinson's Disease (PD).

[0007] In a second aspect, the present disclosure provides a method for assessing severity or risk of a neurodegenerative disorder in two subjects, comprising: (i) comparing the first subject's plasma or serum or whole blood level of Nell-1 protein with the second subject's plasma or serum or whole blood level of Nell-1 protein; (ii) detecting the second subject's plasma or serum or whole blood level of Nell-1 protein lower than the first subject's plasma or serum or whole blood level of the Nell-1 protein; and (iii) determining the second subject as having lower severity or lower risk of the neurodegenerative disorder than the first subject. In a second example, the present disclosure provides a method for assessing the level of comparative risk for a neurodegenerative disorder such as AD, MCI, or PD among two or more subjects. The method includes these steps: first, comparing the first subject's plasma or serum or whole blood level of Nell-1 protein with the second subject's plasma or serum or whole blood level of Nell-1 protein; second, detecting the second subject's plasma or serum or whole blood level of Nell-1 protein lower than the first subject's plasma or serum or whole blood level of the Nell-1 protein; and third, determining the second subject as having lower severity or lower risk of the neurodegenerative disorder than the first subject. In some examples, the neurodegenerative disorder for which the risk is assessed is Alzheimer's Disease (AD). In some examples, the neurodegenerative disorder for which the risk is assessed is Mild Cognitive Impairment (MCI) or Parkinson's Disease (PD). In some examples, the claimed method further comprises, prior to the first step, measuring the plasma or serum or whole blood level of Nell-1 protein. In some examples, the method as mentioned above, further comprises, prior to the measuring step, a step of obtaining a plasma or serum or whole blood sample from the subject.

[0008] In a third aspect, the present disclosure provides a kit for diagnosing a neurodegenerative disorder or assessing severity or risk of a neurodegenerative disorder in a subject, comprising a first reagent capable of determining the subject's plasma or serum or whole blood level of Nell-1 protein and optionally a second reagent capable of determining the subject's plasma or serum or whole blood level of phosphorylated-Tau-181 (pTau181) or neurofilament light polypeptide (NfL). In a third example, the present disclosure provides a kit for assessing risk for neurodegenerative disorders such as AD, MCI, and PD in a subject or for assessing therapeutic efficacy of a treatment regimen for these neurodegenerative disorders. The kit includes a first container containing a first reagent capable of determining the subject's plasma or serum or whole blood level of Nell-1 protein, and, optionally, a second container containing a second reagent capable of determining the subject's

plasma or serum or whole blood level of phosphorylated-Tau-181 (pTau181) or neurofilament light polypeptide (NfL). In some examples, the kit includes reagents capable of determining the subject's plasma or serum or whole blood level of two proteins pTau181 and NfL. In some examples, the kit further includes a standard control reflecting the level of Nell-1 protein found in the plasma or serum or whole blood of an average healthy subject not suffering from and not having an increased risk for the neurodegenerative disorder. In some examples, the kit described above or herein is intended for assessing the risk of or the therapeutic efficacy of a treatment regimen for the neurodegenerative disorder of Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), or Parkinson's Disease (PD).

[0009] In a fourth aspect, the present disclosure provides a use of an effective amount of (1) a Nell-1 enhancing agent that enhances Nell-1 protein expression or activity; or (2) a Nell-1 receptor enhancing agent that enhances Nell-1 receptor expression or activity in the manufacture of a medicament for treating or reducing risk for a neurodegenerative disorder. In a fourth example, the present disclosure provides a method for treating a neurodegenerative disorder or for reducing the risk for such a disorder. The method includes the step of administering to a subject in need thereof an effective amount of (1) a Nell-1 enhancing agent that enhances Nell-1 protein expression or activity; or (2) a Nell-1 receptor enhancing agent that enhances Nell-1 receptor expression or activity. In some examples, the Nell-1 enhancing agent is Nell-1 protein or a nucleic acid encoding the Nell-1 protein. In some examples, the Nell-1 enhancing agent is a Nell-1 receptor, a nucleic acid encoding the Nell-1 receptor, or an agonist of the Nell-1 receptor. In some examples, the step of administration comprises brain-targeting delivery of the Nell-1 enhancing agent or the Nell-1 receptor enhancing agent. In some examples, where a nucleic acid encoding Nell-1 protein or the Nell-1 receptor is administered, the nucleic acid is formulated in a lipid nanoparticle composition for delivery. In some examples, the nucleic acid encoding Nell-1 protein or the Nell-1 receptor is introduced by gene editing. In some examples, this treatment method as claimed further comprises, before and/or after the administering step, measuring the subject's plasma or serum or whole blood level of Nell-1 protein. In some examples, the claimed method further comprises, prior to the measuring step or steps, obtaining a plasma or serum or whole blood sample from the subject. In some examples of this treatment method, the neurodegenerative disorder being treated is Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), or Parkinson's Disease (PD).

[0010] In a fifth aspect, the present disclosure provides a method for assessing efficacy of a therapeutic agent for treating a neurodegenerative disorder in a subject, comprising: (1) comparing the subject's plasma or serum or whole blood level of Nell-1 protein before and after the therapeutic agent is to be administered to the subject; (2) detecting an increase in the subject's plasma or serum or whole blood level of Nell-1 protein after the therapeutic agent is to be administered; and (3) determining the therapeutic agent as effective for treating the neurodegenerative disorder. In a fifth example, the present disclosure provides a method for assessing efficacy of a therapeutic agent for treating a neurodegenerative disorder in a subject. The method includes these steps: (1) comparing the subject's plasma or serum or whole blood level of Nell-1 protein before and after administration of the therapeutic agent to the subject; (2) detecting an increase in the subject's plasma or serum or whole blood level of Nell-1 protein after administration of the therapeutic agent; and (3) determining the therapeutic agent as effective for treating the neurodegenerative disorder. In some

examples, the neurodegenerative disorder being treated is Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), or Parkinson's Disease (PD). In some examples, the claimed method further comprises, prior to step (1), measuring the plasma or serum or whole blood level of Nell-1 protein before and after administration. In some examples, the method further comprises, prior to the measuring step, obtaining a plasma or serum or whole blood sample from the subject before and after administration.

[0011] In some examples of the methods described above and herein, the subject or subjects being tested for a neurodegenerative disorder (such as AD, MCI, or PD) or its risk or being treated for a neurodegenerative disorder or its risk is/are of the Chinese descent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Figure 1. Decreased Nell-1 protein level in blood differentiates patients with AD from cognitively normal subjects. **Figure 1a:** Box-and-whisker plot showing the individual plasma Nell-1 levels of cognitively normal (CN) subjects and patients with Alzheimer's disease (AD) from the Hong Kong Chinese cohort ($n = 119$ CN + 152 AD). Statistical analysis was performed by linear regression adjusting for age, sex, and history of cardiovascular disease; $\beta = -0.158$, $*P < 0.05$. **Figure 1b:** Receiver operating characteristic (ROC) curve and corresponding area under the curve (AUC) representing the performance of plasma Nell-1 level in classifying AD phenotype in the Hong Kong Chinese cohort.

[0013] Figure 2. Decreased Nell-1 protein level in blood differentiates patients with MCI from cognitively normal subjects. **Figure 2a:** Box-and-whisker plot showing the individual plasma Nell-1 levels of cognitively normal (CN) subjects and patients with mild cognitive impairment (MCI) from the Hong Kong Chinese cohort ($n = 119$ CN + 109 MCI). Statistical analysis was performed by linear regression adjusting for age, sex, and history of cardiovascular disease; $\beta = -0.187$, $*P < 0.05$. **Figure 2b:** Receiver operating characteristic (ROC) curve and corresponding area under the curve (AUC) representing the performance of plasma Nell-1 level in classifying MCI phenotype in the Hong Kong Chinese cohort.

[0014] Figure 3. Decreased Nell-1 protein level in blood differentiates patients with PD from cognitively normal subjects. **Figure 3a:** Box-and-whisker plot showing the individual plasma Nell-1 levels of cognitively normal (CN) subjects and patients with Parkinson's disease (PD) from the Hong Kong Chinese cohort ($n = 119$ CN + 39 PD). Statistical analysis was performed by linear regression adjusting for age and sex; $\beta = -0.370$, $***P < 0.001$. **Figure 3b:** Receiver operating characteristic (ROC) curve and

WHAT IS CLAIMED IS:

1. A method for diagnosing, monitoring, or assessing risk for a neurodegenerative disorder in a subject, comprising:
 - (1) comparing the subject's plasma or serum or whole blood level of Nell-1 protein with a standard control level of Nell-1 protein found in the plasma or serum or whole blood of an average healthy subject not suffering from or at increased risk for the neurodegenerative disorder; and
 - (2) detecting a decrease in the subject's plasma or serum or whole blood level of Nell-1 protein from the standard control level, and determining the subject as suffering from or having an increased risk for the neurodegenerative disorder; or
 - (3) detecting no decrease in the subject's plasma or serum or whole blood level of Nell-1 protein from the standard control level, and determining the subject as not suffering from or having no increased risk for the neurodegenerative disorder.
2. The method of claim 1, further comprising, prior to step (1), measuring the plasma or serum or whole blood level of Nell-1 protein.
3. The method of claim 2, further comprising, prior to the measuring step, a plasma or serum or whole blood sample which is to be obtained from the subject.
4. The method of claim 1, further comprising, after step (3), comparing the subject's plasma or serum or whole blood level of Nell-1 protein as measured at a later time with the subject's plasma or serum or whole blood level of Nell-1 protein in step (1), wherein a higher plasma or serum or whole blood level of Nell-1 protein as measured at the later time indicates improvement of the neurodegenerative disorder, and wherein a lower plasma or serum or whole blood level of Nell-1 protein as measured at the later time indicates deterioration of the neurodegenerative disorder.
5. The method of claim 4, wherein between step (3) and the later time the subject has been administered a therapeutic agent intended for treating the neurodegenerative disorder.

6. The method of any one of claims 1-5, wherein the neurodegenerative disorder is Alzheimer's Disease (AD).

7. The method of any one of claims 1-5, wherein the neurodegenerative disorder is Mild Cognitive Impairment (MCI) or Parkinson's Disease (PD).

8. A method for assessing severity or risk of a neurodegenerative disorder in two subjects, comprising:

(i) comparing the first subject's plasma or serum or whole blood level of Nell-1 protein with the second subject's plasma or serum or whole blood level of Nell-1 protein;

(ii) detecting the second subject's plasma or serum or whole blood level of Nell-1 protein lower than the first subject's plasma or serum or whole blood level of the Nell-1 protein; and

(iii) determining the second subject as having lower severity or lower risk of the neurodegenerative disorder than the first subject.

9. The method of claim 8, wherein the neurodegenerative disorder is Alzheimer's Disease (AD).

10. The method of claim 8, wherein the neurodegenerative disorder is Mild Cognitive Impairment (MCI) or Parkinson's Disease (PD).

11. The method of claim 8 or 9, further comprising, prior to step (i), measuring the plasma or serum or whole blood level of Nell-1 protein.

12. The method of claim 11, further comprising, prior to the measuring step, a plasma or serum or whole blood sample which is to be obtained from the subject.

13. A kit for diagnosing a neurodegenerative disorder or assessing severity or risk of a neurodegenerative disorder in a subject, comprising a first reagent capable of determining the subject's plasma or serum or whole blood level of Nell-1 protein and optionally a second reagent capable of determining the subject's plasma or serum or whole blood level of phosphorylated-Tau-181 (pTau181) or neurofilament light polypeptide (NFL).

14. The kit of claim 12, comprising reagents capable of determining the subject's plasma or serum or whole blood level of pTau181 and NfL.

15. The kit of claim 12, further comprising a standard control reflecting the level of Nell-1 protein found in the plasma or serum or whole blood of an average healthy subject not suffering from and not having an increased risk for the neurodegenerative disorder.

16. The kit of any one of claims 12-15, wherein the neurodegenerative disorder is Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), or Parkinson's Disease (PD).

17. Use of an effective amount of (1) a Nell-1 enhancing agent that enhances Nell-1 protein expression or activity; or (2) a Nell-1 receptor enhancing agent that enhances Nell-1 receptor expression or activity in the manufacture of a medicament for treating or reducing risk for a neurodegenerative disorder.

18. The use of claim 17, wherein the enhancing Nell-1 agent is Nell-1 protein or a nucleic acid encoding Nell-1 protein.

19. The use of claim 17, wherein the enhancing Nell-1 agent is a Nell-1 receptor, a nucleic acid encoding the Nell-1 receptor, or an agonist of the Nell-1 receptor.

20. The use of claim 17, wherein the Nell-1 enhancing agent or the Nell-1 receptor enhancing agent is to be delivered to the brain.

21. The use of claim 19 or 20, wherein the nucleic acid encoding Nell-1 protein or the Nell-1 receptor is formulated in a lipid nanoparticle composition for delivery.

22. The use of claim 19 or 20, wherein the nucleic acid encoding Nell-1 protein or the Nell-1 receptor is introduced by gene editing.

23. The use of any one of claims 17-22, further comprising, measuring the subject's plasma or serum or whole blood level of Nell-1 protein before and/or after the Nell-1 enhancing agent or Nell-1 receptor enhancing agent is to be administered.

24. The use of claim 23, further comprising, prior to the measuring step or steps, a plasma or serum or whole blood sample which is to be obtained from the subject.

25. The use of any one of claims 17-24, wherein the neurodegenerative disorder is Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), or Parkinson's Disease (PD).

26. A method for assessing efficacy of a therapeutic agent for treating a neurodegenerative disorder in a subject, comprising:

- (1) comparing the subject's plasma or serum or whole blood level of Nell-1 protein before and after the therapeutic agent is to be administered to the subject;
- (2) detecting an increase in the subject's plasma or serum or whole blood level of Nell-1 protein after the therapeutic agent is to be administered; and
- (3) determining the therapeutic agent as effective for treating the neurodegenerative disorder.

27. The method of claim 26, wherein the neurodegenerative disorder is Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), or Parkinson's Disease (PD).

28. The method of claim 26 or 27, further comprising, prior to step (1), measuring the plasma or serum or whole blood level of Nell-1 protein before and after the therapeutic agent is to be administered.

29. The method of claim 28, further comprising, prior to the measuring step, a plasma or serum or whole blood sample which is to be obtained from the subject before and after the therapeutic agent is to be administered.

30. The method of any one of claims 1-12 and 17-29, wherein the subject(s) is/are of Chinese descent.