

GG-FXN (SEQ ID NO: 11) is described in US Provisional Patent Application No. 62/891,029, the entire contents of each of which are hereby incorporated herein by reference.

To facilitate development of FXN replacement therapies, new reliable methods are needed to accurately and precisely measure level of FXN and FXN fusion proteins in various biological fluids and tissues of a subject.

SUMMARY

In one aspect, the present disclosure refers to a method for determining the amount of unprocessed frataxin (FXN) fusion protein in a sample, said method comprising determining the amount of at least one peptide in said sample by using mass spectrometry (MS), wherein said unprocessed FXN fusion protein comprises full length FXN having the sequence of SEQ ID NO: 1 and a cell penetrating peptide (CPP) selected from HIV-TAT having the sequence of SEQ ID NO: 4 and HIV-TAT+M having the sequence of SEQ ID NO: 15; wherein said at least one peptide is selected from the group consisting of: a) a peptide comprising the amino acid sequence SGTLGHPGSLDETTYER (SEQ ID NO: 13); and b) a peptide comprising the amino acid sequence LGGDLGTYVINK (SEQ ID NO: 14); wherein said method comprises purifying by immunocapture the unprocessed FXN fusion protein from said sample prior to MS; wherein said immunocapture is carried out using a binding protein comprising an antigen binding domain, wherein said binding protein is capable of binding a TAT protein transduction domain.

In another aspect, the present disclosure refers to a method for determining the amount of frataxin (FXN) in a tissue sample, said method comprising determining the amount of at least one peptide derived from said FXN in said sample by using mass spectrometry (MS), wherein said at least one peptide is selected from the group consisting of: a) a peptide comprising the amino acid sequence SGTLGHPGSLDETTYER (SEQ ID NO: 13); and b) a peptide comprising the amino acid sequence LGGDLGTYVINK (SEQ ID NO: 14); wherein said tissue sample comprises a buccal swab or a skin biopsy; wherein the method is characterized by at least one of the following: the method has a precision as measured by % coefficient of variation (%CV) of 20% or less; and the method has an accuracy of 80% to 120%.

In one example, the present disclosure provides a method for determining the amount of unprocessed frataxin (FXN) fusion protein in a sample, said method comprising determining the amount of a peptide in said sample by using mass spectrometry (MS),

wherein said peptide comprises, or consists of, the amino acid sequence GGMWTLGR (SEQ ID NO: 12); and

wherein said unprocessed FXN fusion protein comprises, or consists of, from N-terminus to C-terminus, a cell penetrating peptide (CPP); a mitochondrial targeting sequence (MTS) comprising, or consisting of, SEQ ID NO: 3; and FXN comprising, or consisting of, SEQ ID NO: 2.

In one example, said sample is obtained from a subject being administered the unprocessed FXN fusion protein.

In one example, said CPP comprises, or consists of, HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15).

In one example, said unprocessed FXN fusion protein is TAT-GG-FXN (SEQ ID NO: 11).

In one example, said method comprises purifying by immunocapture the unprocessed FXN fusion protein from said sample prior to MS.

In one example, said method comprises the following steps:

CLAIMS

1. A method for determining the amount of unprocessed frataxin (FXN) fusion protein in a sample, said method comprising determining the amount of at least one peptide in said sample by using mass spectrometry (MS),

wherein said unprocessed FXN fusion protein comprises full length FXN having the sequence of SEQ ID NO: 1 and a cell penetrating peptide (CPP) selected from HIV-TAT having the sequence of SEQ ID NO: 4 and HIV-TAT+M having the sequence of SEQ ID NO: 15;

wherein said at least one peptide is selected from the group consisting of:

a) a peptide comprising the amino acid sequence
SGTLGHPGSLDETTYER (SEQ ID NO: 13); and

b) a peptide comprising the amino acid sequence LGGDLGTYVINK
(SEQ ID NO: 14);

wherein said method comprises purifying by immunocapture the unprocessed FXN fusion protein from said sample prior to MS;

wherein said immunocapture is carried out using a binding protein comprising an antigen binding domain, wherein said binding protein is capable of binding a TAT protein transduction domain.

2. The method of claim 1, wherein said sample is to be obtained from a subject being administered the unprocessed FXN fusion protein.

3. The method of claim 1 or 2, wherein said method comprises the following steps:

a) purifying by immunocapture the unprocessed FXN fusion protein from said sample, thereby obtaining immunocaptured complexes comprising the unprocessed FXN fusion protein;

b) subjecting said immunocaptured complexes comprising the unprocessed FXN fusion protein to digestion by trypsin, thereby producing the peptide comprising SEQ ID NO: 13 and/or the peptide comprising SEQ ID NO: 14; and

c) determining the amount of the peptide comprising SEQ ID NO: 13 and/or the peptide comprising SEQ ID NO: 14 by liquid chromatography and tandem mass spectrometry (LC/MS-MS).

4. The method of claim 3, wherein in part c) the liquid chromatography (LC) comprises reversed-phase chromatography.

5. The method of claim 3 or 4, wherein the MS-MS comprises monitoring the transition 607.3 → 669.3 for the peptide comprising SEQ ID NO: 13 and the transition 625.3 → 794.4 for the peptide comprising SEQ ID NO: 14.

6. The method of any one of claims 1-5, wherein the sample is a tissue sample.

7. The method of claim 6, wherein the tissue sample comprises a buccal swab or a skin biopsy.

8. The method of any one of claims 1-5, wherein the sample is derived from the blood of the subject.

9. The method of claim 8, wherein the sample comprises platelets or wherein the sample is a plasma sample.

10. The method of any one of claims 1-9, wherein the method has a precision as measured by % coefficient of variation (%CV) of 20% or less.

11. The method of any one of claims 1-10, wherein the method has an accuracy of 80% to 120%.

12. The method of any one of claims 1-11, wherein the method is linear for concentrations of unprocessed FXN fusion protein ranging from 0.250 ng/mL to 25.000 ng/mL.

13. A method for determining the amount of frataxin (FXN) in a tissue sample, said method comprising determining the amount of at least one peptide derived from said FXN in said sample by using mass spectrometry (MS),

wherein said at least one peptide is selected from the group consisting of:

a) a peptide comprising the amino acid sequence
SGTLGHPGSLDETTYER (SEQ ID NO: 13); and

b) a peptide comprising the amino acid sequence LGGDLGTYVINK
(SEQ ID NO: 14);

wherein said tissue sample comprises a buccal swab or a skin biopsy;

wherein the method is characterized by at least one of the following:

the method has a precision as measured by % coefficient of variation (%CV)
of 20% or less; and

the method has an accuracy of 80% to 120%.

14. The method of claim 13, wherein said sample is to be obtained from a subject.

15. The method of claim 14, wherein said subject is to be administered unprocessed FXN fusion protein.

16. The method of any one of claims 1-12 or 15, wherein said unprocessed FXN fusion protein comprises, from N-terminus to C-terminus, a cell penetrating peptide (CPP), a mitochondrial targeting sequence (MTS) of SEQ ID NO: 3 and FXN.

17. The method of claim 16, wherein said CPP comprises HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15).

18. The method of claim 17, wherein said unprocessed FXN fusion protein is TAT-GG-FXN (SEQ ID NO: 11).

19. The method of any one of claims 13-18, wherein said method comprises purifying by immunocapture the FXN from said sample prior to MS.

20. The method of any one of claims 13-19, wherein said method comprises the following steps:

a) purifying by immunocapture the FXN from said sample, thereby obtaining immunocaptured complexes comprising FXN;

b) subjecting said immunocaptured complexes comprising FXN to digestion by trypsin, thereby producing the peptide comprising SEQ ID NO: 13 and/or the peptide comprising SEQ ID NO: 14; and

c) determining the amount of at least one peptide selected from the group consisting of the peptide comprising SEQ ID NO: 13 and/or the peptide comprising SEQ ID NO: 14 by liquid chromatography and tandem mass spectrometry (LC/MS-MS).

21. The method of claim 20, wherein in part c) the liquid chromatography (LC) comprises reversed-phase chromatography.

22. The method of claim 20 or 21, wherein the tandem mass spectrometry (MS-MS) comprises monitoring the transition 607.3 → 669.3 for the peptide comprising SEQ ID NO: 13 and the transition 625.3 → 794.4 for the peptide comprising SEQ ID NO: 14.

23. The method of any one of claims 19-22, wherein the immunocapture is carried out using a binding protein comprising an antigen binding domain that specifically binds FXN.

24. The method of any one of claims 19-22, wherein said FXN is a part of an FXN fusion protein also comprising HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15) and wherein said binding protein specifically binds the HIV-TAT.