

[0010] Against this background, among a large number of phosphorylated sites in the tau protein which can undergo abnormal phosphorylation in the conditions of Alzheimer's disease, the present inventors focused on the phosphorylated site of the serine residue at position 413 (Ser413), and succeeded in producing a polyclonal rabbit antibody and a monoclonal mouse antibody which are specific to the Ser413-phosphorylated tau. The present inventors also administered the monoclonal mouse antibodies to model mice of cognitive disorders which showed abnormal expression of phosphorylated tau proteins, and confirmed that improvement in cognitive functions was observed (WO2013/180238A).

[0010a] In one aspect, the present disclosure refers to an anti-pSer413 tau antibody comprising:

- (A) ~~(i) a variable heavy (VH) domain having an amino acid sequence of SEQ ID NO:20, and~~
~~—— (ii) a variable light (VL) domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~
~~—— (B) (i) a VH domain having an amino acid sequence of SEQ ID NO:22, and~~
~~—— (ii) a VL domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~
~~—— (C) (i) a VH domain having an amino acid sequence of SEQ ID NO:24, and —— (ii) a VL domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~
~~—— (D) (i) a VH domain having an amino acid sequence of SEQ ID NO:26, and~~
~~—— (ii) a VL domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~
~~—— (E) (i) a VH domain having an amino acid sequence of SEQ ID NO:28, and~~
~~—— (ii) a VL domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~

~~(F)~~ (i) a VH domain having an amino acid sequence of SEQ ID NO:116, and
(ii) a VL domain having an amino acid sequence of ~~SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42~~, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114; or

~~(GB)~~ (i) a VH domain having an amino acid sequence of SEQ ID NO:117, and
(ii) a VL domain having an amino acid sequence of ~~SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42~~, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114.

[0010b] In another aspect, the present disclosure refers to a humanized antibody, or fragment or derivative thereof, which causes an antigen-antibody reaction with a tau protein or a tau peptide phosphorylated at least on an amino acid residue corresponding to Ser413 of SEQ ID NO: 1, comprising

(A) a VH domain having:

- (i) an amino acid sequence of SEQ ID NO:30 (VH65),
- (ii) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Ala with Thr at position 28,
- (iii) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Asn with Ser at position 30,
- (iv) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Val with Gly at position 49,
- (v) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Ala with Asp at position 64, or
- (vi) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Gln with Lys at position 78; and

(B) a VL domain having:

- (i) an amino acid sequence of SEQ ID NO:38,
- (ii) an amino acid sequence of SEQ ID NO:38, comprising a substitution of Asp with Glu at position 17,
- (iii) an amino acid sequence of SEQ ID NO:38, comprising a substitution of Ser with Asn at position 28,

CLAIMS

What Is Claimed Is:

1. An anti-pSer413 tau antibody comprising:

(A) ~~(i) a variable heavy (VH) domain having an amino acid sequence of SEQ ID NO:20,~~
and

~~—— (ii) a variable light (VL) domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~

~~—— (B) (i) a VH domain having an amino acid sequence of SEQ ID NO:22, and~~

~~—— (ii) a VL domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~

~~—— (C) (i) a VH domain having an amino acid sequence of SEQ ID NO:24, and~~

~~—— (ii) a VL domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~

~~—— (D) (i) a VH domain having an amino acid sequence of SEQ ID NO:26, and~~

~~—— (ii) a VL domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~

~~—— (E) (i) a VH domain having an amino acid sequence of SEQ ID NO:28, and~~

~~—— (ii) a VL domain having an amino acid sequence of SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105,~~

~~SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114;~~

~~(F)~~ (i) a VH domain having an amino acid sequence of SEQ ID NO:116, and

~~_____~~ (ii) a VL domain having an amino acid sequence of ~~SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42,~~ SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114; or

~~(BG)~~ (i) a VH domain having an amino acid sequence of SEQ ID NO:117, and

(ii) a VL domain having an amino acid sequence of ~~SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:42,~~ SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, or SEQ ID NO:114.

2. The anti-pSer413 tau antibody of claim 1, wherein the ratio of said antibody's binding affinity to a phosphorylated peptide of SEQ ID NO:8 to said antibody's binding affinity to a non-phosphorylated peptide of SEQ ID NO:69 is at least about 40 to 1.

3. The anti-pSer413 tau antibody of claim 1, wherein said antibody comprises a heavy chain constant domain having an amino acid sequence of SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142 or SEQ ID NO:143.

4. The anti-pSer413 tau antibody of claim 1 wherein said antibody comprises a light chain constant domain having an amino acid sequence of SEQ ID NO:79 or SEQ ID NO:80.

5. The anti-pSer413 tau antibody of claim 1, wherein said antibody comprises:

(A) (i) a heavy chain having an amino acid sequence of SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID

NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, or SEQ ID NO:161, and

(ii) a light chain having an amino acid sequence of SEQ ID NO:184; or

(B) (i) a heavy chain having an amino acid sequence of SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, or SEQ ID NO:161, and

(ii) a light chain having an amino acid sequence of SEQ ID NO:185.

6. A humanized antibody, or fragment or derivative thereof, which causes an antigen-antibody reaction with a tau protein or a tau peptide phosphorylated at least on an amino acid residue corresponding to Ser413 of SEQ ID NO: 1, comprising

(A) a VH domain having:

(i) an amino acid sequence of SEQ ID NO:30 (VH65),

(ii) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Ala with Thr at position 28,

(iii) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Asn with Ser at position 30,

(iv) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Val with Gly at position 49,

(v) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Ala with Asp at position 64, or

(vi) an amino acid sequence of SEQ ID NO:30 comprising a substitution of Gln with Lys at position 78; and

(B) a VL domain having:

(i) an amino acid sequence of SEQ ID NO:38,

(ii) an amino acid sequence of SEQ ID NO:38, comprising a substitution of Asp with Glu at position 17,

- (iii) an amino acid sequence of SEQ ID NO:38, comprising a substitution of Ser with Asn at position 28,
- (iv) an amino acid sequence of SEQ ID NO:38, comprising a substitution of Gln with Leu at position 42,
- (v) an amino acid sequence of SEQ ID NO:38, comprising a substitution of Gln with Arg at position 50, or
- (vi) an amino acid sequence of SEQ ID NO:38, comprising a substitution of Arg with Leu at position 51.

7. An anti-pSer413 tau antibody comprising a VH domain having an amino acid sequence of SEQ ID NO:116, and a VL domain having an amino acid sequence of SEQ ID NO:114.

8. The anti-pSer413 tau antibody of claim 7, wherein the ratio of said antibody's binding affinity to a phosphorylated peptide of SEQ ID NO:8 to said antibody's binding affinity to a non-phosphorylated peptide of SEQ ID NO:69 is at least about 40 to 1.

9. The anti-pSer413 tau antibody of claim 7, wherein said antibody comprises a heavy chain constant domain having an amino acid sequence of SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142 or SEQ ID NO:143.

10. The anti-pSer413 tau antibody of claim 7, wherein said antibody comprises a light chain constant domain having an amino acid sequence of SEQ ID NO:79 or SEQ ID NO:80.

11. The anti-pSer413 tau antibody of claim 7, comprising a light chain having an amino acid sequence of SEQ ID NO:184.

12. The anti-pSer413 tau antibody of claim 11, comprising a heavy chain having an amino acid sequence of SEQ ID NO:144, and a light chain having an amino acid sequence of SEQ ID NO:184.

13. The anti-pSer413 tau antibody of claim 11, comprising a heavy chain having an amino acid sequence of SEQ ID NO:152, and a light chain having an amino acid sequence of SEQ ID NO:184.

14. Use of the anti-pSer413 tau antibody of any one of claims 1-5 and 7-13 in the manufacture of a medicament for treating tauopathy in a subject.

15. The use of claim 14, wherein the tauopathy is selected from a group consisting of Alzheimer's disease, corticobasal degeneration, progressive supranuclear palsy, Pick's disease, argyrophilic grain dementia (argyrophilic grain disease), multiple system tauopathy with presenile dementia (MSTD), frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), dementia with neurofibrillary tangles, diffuse neurofibrillary tangle with calcification (DNTC), white matter tauopathy with globular glial inclusions (WMT-GGI), frontotemporal lobar degeneration with tau pathology (FTLD-tau), Economo's encephalitis sequela, subacute sclerosing panencephalitis, and boxer's encephalopathy.

16. A nucleic acid composition comprising:

- (i) a first nucleic acid encoding a heavy chain having an amino acid sequence of SEQ ID NO:144; SEQ ID NO:145; SEQ ID NO:146; SEQ ID NO:147; SEQ ID NO:148; SEQ ID NO:149; SEQ ID NO:150; SEQ ID NO:151; SEQ ID NO:152; SEQ ID NO:153; SEQ ID NO:154; SEQ ID NO:155; SEQ ID NO:156; SEQ ID NO:157; SEQ ID NO:158; SEQ ID NO:159; SEQ ID NO:160, or SEQ ID NO:161; and
- (ii) a second nucleic acid encoding a light chain having an amino acid sequence of SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:165, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID

NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, or SEQ ID NO:185.

17. The nucleic acid composition of claim 16, comprising: (i) a first nucleic acid encoding a heavy chain having an amino acid sequence of SEQ ID NO:144; and (ii) a second nucleic acid encoding a light chain having an amino acid sequence of SEQ ID NO:184.

18. The nucleic acid composition of claim 16, comprising: (i) a first nucleic acid encoding a heavy chain having an amino acid sequence of SEQ ID NO:152; and (ii) a second nucleic acid encoding a light chain having an amino acid sequence of SEQ ID NO:184.

19. The nucleic acid composition of any one of claims 16-18, wherein the first nucleic acid is contained in a first expression vector, and the second nucleic acid is contained in a second expression vector.

20. The nucleic acid composition of any one of claims 16-18, wherein the first nucleic acid and the second nucleic acid are contained in one expression vector.

21. A host cell comprising the nucleic acid composition of any one of claims 16-18.

22. A method of making an anti-pSer413 tau antibody, comprising culturing the host cell of claim 21 under conditions wherein the antibody is expressed, and recovering the antibody.