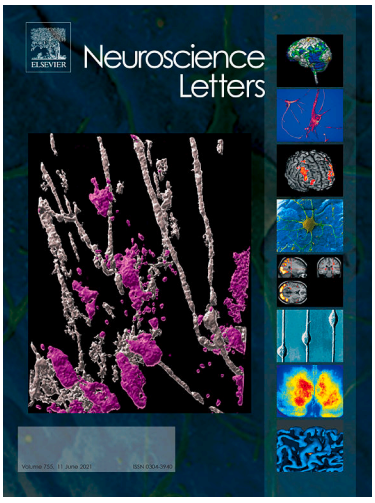
Journal Pre-proofs



Alzheimer's disease susceptibility locus in *CD2AP* is associated with increased cerebrospinal fluid tau levels in mild cognitive impairment

Yan-Yan Xue, Yi-He Chen, Rong-Rong Lin, Hui-Fen Huang, Zhi-Ying Wu, Qing-Qing Tao

PII: S0304-3940(21)00798-9
DOI: https://doi.org/10.1016/j.neulet.2021.136419
Reference: NSL 136419

To appear in: *Neuroscience Letters*

Received Date: 21 October 2021
Revised Date: 10 December 2021
Accepted Date: 21 December 2021

Please cite this article as: Y-Y. Xue, Y-H. Chen, R-R. Lin, H-F. Huang, Z-Y. Wu, Q-Q. Tao, Alzheimer's disease susceptibility locus in *CD2AP* is associated with increased cerebrospinal fluid tau levels in mild cognitive impairment, *Neuroscience Letters* (2021), doi: https://doi.org/10.1016/j.neulet.2021.136419

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Elsevier B.V. All rights reserved.

Title page

Title

Alzheimer's disease susceptibility locus in *CD2AP* is associated with increased cerebrospinal fluid tau levels in mild cognitive impairment

Names of authors and affiliations

Yan-Yan Xue, Yi-He Chen, Rong-Rong Lin, Hui-Fen Huang, Zhi-Ying Wu*, Qing-Qing Tao*, Alzheimer's Disease Neuroimaging Initiative

Department of Neurology and Research Center of Neurology in Second Affiliated Hospital, Key Laboratory of Medical Neurobiology of Zhejiang Province, Zhejiang University School of Medicine, Hangzhou, China

***Corresponding author**

Correspondence and reprint requests may be directed to Dr. Qing-Qing Tao (e-mail: qingqingtao@zju.edu.cn) and Prof. Zhi-Ying Wu (e-mail: zhiyingwu@zju.edu.cn)

Abstract

Introduction: Rs9296559 within CD2-associated protein (*CD2AP*) has been identified as a susceptibility locus for Alzheimer's disease (AD). Recent studies indicated that CD2AP functioned as a regulator of endocytic trafficking to modulate the β-amyloid (Aβ) generation in neurons. Moreover, knockdown of cindr, the Drosophila ortholog of *CD2AP*, enhanced tau-induced neurodegeneration, implying CD2AP also participated in tau pathology. However, the role of rs9296559 in regulating Aβ and tau metabolism in AD was still unclear.

Methods: Here, the associations of rs9296559 with CSF $A\beta_{1-42}$, p-tau, and t-tau were performed using a linear regression model in a total of 543 cognitive normal (CN), mild cognitive impairment (MCI), and AD subjects from the Alzheimer's disease Neuroimaging Initiative (ADNI) cohort. The results were replicated in an independent

Journal Pre-proofs

cohort consisting of 198 Chinese subjects recruited from our hospital.

Results: In the ADNI cohort, CC+TC genotypes significantly increased CSF t-tau and p-tau levels in MCI patients but did not alter CSF tau levels in AD. This association was also observed in the replication cohort. Moreover, there was no association between rs9296559 and CSF $A\beta_{1-42}$ level at different disease statuses in the two cohorts.

Conclusion: Our findings showed that rs9296559 was associated with higher CSF t-tau and p-tau levels in MCI, supporting that CD2AP modified AD risk by altering tau-related neurodegeneration in the early stage of the AD continuum. To the best of our knowledge, this is the first study to evaluate the association between *CD2AP* genotypes and AD CSF biomarkers.

Keywords: Alzheimer's disease, CD2AP, rs9296559, tau, cerebrospinal fluid tau

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder often characterized by progressive loss of memory and cognitive decline. It has been documented that the deposition of β-amyloid (Aβ) and tau pathology in the brain existed as early as more than ten years before the AD clinical symptoms appeared [1]. Owing to the tremendous progress of cerebrospinal fluid (CSF) biomarkers and positron emission tomography (PET) analyses, the full trajectory of AD becomes more distinct [2,3].

CD2-associated protein (CD2AP) functions as an adaptor protein consisting of 639 amino acid residues that are expressed abundantly in neurons, immune cells, and epithelial cells [4,5]. The corresponding gene, *CD2AP*, is located on chromosome 6 (6p12.3) and contains 18 exons [6]. We previously reported that the expression of

CD2AP was decreased in peripheral blood lymphocytes in sporadic AD (SAD), implying CD2AP loss of function may be implicated in SAD pathogenesis [7]. CD2AP was reported to affect APP and BACE1 sorting in early endosomes by distinct mechanisms [8]. The loss of function of CD2AP has been linked to the abnormal Aβ levels and Aβ42/Aβ40 ratio in neurons [9]. The fly ortholog of the human *CD2AP*, cindr, was reported to serve as a modulator of tau-mediated neurotoxicity. The loss of function of cindr enhances tau-induced neuronal loss in the adult fly brain [10].

CD2AP rs9296559 SNP has been identified to be significantly associated with AD susceptibility in genome-wide association studies (GWAS) and confirmed by several subsequent studies [11-17]. However, the role of rs9296559 in regulating AD CSF pathogenic proteins was still unclear. To determine the functional consequence of rs9296559 and its possible effects on AD pathogenesis, we analyzed its association with AD CSF biomarkers in cognitive normal (CN), mild cognitive impairment (MCI), and AD subjects from the Alzheimer's disease Neuroimaging Initiative (ADNI) cohort and a replication cohort from China.

Materials and Methods

ADNI dataset

ADNI was initialed in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations (http://adni.loni.usc.edu). ADNI, with Michael W. Weiner, MD as the principal investigator, was approved by the regional ethical committees of all participating institutions, and written informed consent was obtained from all participants.

Participants

Subjects were classified into CN, MCI, and AD at baseline according to the fourth edition of Diagnostic and Statistical Manual of Mental Disease (DSM-IV) and National Institute on Aging and Alzheimer's Association (NIA-AA) research criteria in 2011. In the ADNI cohort, a total of 543 subjects, including 101 CN, 278 MCI and 164 AD, were included. All these participants were enrolled from the ADNI1, ADNI GO, and ADNI2 phases. Participants from ADNI3 phase were excluded owing to the lack of genetic data of rs9296559. In this study, all of the subjects from ADNI were non-Hispanic and white people to avoid bias caused by genetic background differences between ethnicities. To further validate the findings observed in the ADNI cohort, an independent cohort consisting of 198 Chinese individuals was included in this study for further analysis. All participants in our independent cohort were enrolled from Department of Neurology, Second Affiliated Hospital of Zhejiang University School of Medicine, between March 2015 and November 2020. Data workflow was presented in **Figure 1**. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine. Written informed consents were obtained from all participants or legal guardians.

Genotyping

ADNI samples were genotyped using the Illumina Human 610-Quad Bead Chip array (Illumina, Inc., San Diego, CA, USA), including 620,901 SNPs and CNV markers which were employed for genotyping. Here we extracted the genetic data of *CD2AP* (rs9296559) from the ADNI PLINK data format. Samples in our cohort were genotyped using polymerase chain reaction and Sanger sequencing, and corresponding methods have been described previously [7].

CSF $A\beta_{1-42}$, t-tau, and p-tau measurements

Methods for the collection, extraction, and examination of CSF proteins including $A\beta_{1-42}$, $A\beta_{1-40}$, total tau (t-tau) and phosphorylated tau 181(indicated as p-tau later) in our independent cohort have been described previously [18]. The concentrations of CSF proteins could be downloaded from the ADNI database, and detailed steps for measurement have been previously reported [19].

Statistical analysis

All statistical analyses were conducted using SPSS 16.0 and GraphPad Prism7. Nonparametric data with multiple comparisons were analyzed by Dunn's multiple comparisons test followed by Bonferroni's procedure for adjusted *P* values. A multiple linear regression model was used to analyze the association of *CD2AP* (rs9296559) with CSF biomarkers at baseline after corrections for MMSE, age, gender, educational level, and APOE genotypes. For all the tests, we claim that rs9296559 is significantly associated with the levels of CSF biomarkers when *P* values are below 0.05.

Results

Basic characteristics

In the ADNI cohort, there were 543 subjects (CN=101, MCI=278, and AD=164) with both AD CSF pathological proteins and rs9296559 genotypes. This cohort consisted of 324 male and 219 female subjects. The distributions of the rs9296559 genotypes were different among different groups, and a higher frequency of C allele was observed in MCI and AD groups ($P<0.05$). As expected, MCI and AD had a higher frequency for the APOE ε4 allele and performed worse than CN on MMSE. Besides, the levels of CSF $A\beta_{1-42}$, t-tau, and p-tau were different among the three groups, and the MCI and AD groups showed lower CSF $A\beta_{1-42}$, higher p-tau, and t-tau levels than

those in CN **(Figure 2)**. The details of demographic characteristics and distributions of the genotypes are shown in **Table 1**.

CC+TC genotypes of rs9296559 increased CSF t-tau and p-tau levels in MCI

There were no significant differences in CSF $A\beta_{1-42}$, p-tau, and t-tau levels between subjects carrying TT and CC+TC in the CN group **(Figure 3A)**. In the MCI group, rs9296559-C carriers had higher p-tau (32.22±17.09 pg/mL) and t-tau (327.00±158.27 pg/mL) levels than those in TT genotypes (p-tau: 28.10±13.81 pg/mL; t-tau: 287.19±120.06 pg/mL), while they did not differ in CSF Aβ1-42 level (CC+TC: 929.49±533.90 pg/mL; TT: 896.31±477.30 pg/mL) **(Figure 3B)**. However, both CSF $A\beta_{1-42}$ and tau levels showed no significant difference between CC+TC genotypes and TT genotype in the AD group **(Figure 3C)**. After controlling for age, gender, education level, APOE genotypes, and MMSE scores in the multiple linear regression models, CC+TC genotypes significantly increased the level of CSF p-tau (β=0.137, $P<0.05$) and t-tau (β=0.144, $P<0.05$) in the MCI group **(Table 2)**. No significant relationships between the baseline levels of CSF $A\beta_{1-42}$, t-tau, and p-tau and *CD2AP* rs9296559 were observed in the CN and AD groups ($P>0.05$) **(Table 2)**. Taken together, CC+TC genotypes of rs9296559 were associated with higher CSF t-tau and p-tau levels in MCI but had no significant influence on $A\beta_{1-42}$ regardless of different disease status in the AD continuum.

Association between SNP rs9296559 and CSF tau levels in an independent cohort

To further validate the relationship between rs9296559 variants and CSF p-tau and t-tau levels, we measured CSF tau concentrations and genotyped the rs9296559 SNP in an independent cohort of 198 adult participants. After clinical evaluation and sample collection, 55 participants were diagnosed with CN, 33 with MCI and 110 with AD. In this cohort, the frequency of the minor (C) allele of rs9296559 was

Journal Pre-proofs

16.2% in our cohort, almost half that in ADNI. The details of demographic characteristics and distributions of the genotypes are shown in **Table 3**. The levels of CSF Aβ1-42, t-tau, and p-tau were different among the three groups, and the MCI and AD groups showed lower CSF Aβ1-42, higher p-tau, and t-tau levels than those in CN (**Figure 4**). There was no significant difference of CSF Aβ1-42, p-tau, and t-tau levels between subjects carrying TT and CC+TC in CN and AD groups while p-tau level was significant elevated in subjects carrying CC+TC ($P < 0.05$) in the MCI group (**Figure 5**). However, t-tau level was insignificantly increased in subjects carrying CC+TC ($P=0.06$) (**Figure 5B**). To rule out the confounding factors, a linear regression model was performed. In the MCI group, rs9296559 was significantly associated with both CSF p-tau ($\beta=0.564$, $P<0.05$) and t-tau ($\beta=0.483$, $P<0.05$) levels in a linear regression model adjusted for age, sex, education, MMSE, and APOE. As for $A\beta_{1-42}$ level, rs9296559 showed no association with it in MCI ($\beta=0.203$, $P=0.279$). Consistent with the above findings in CN and AD, there was little difference in the level of $A\beta_{1-42}$, t-tau, and p-tau between in CC+TC group and TT group, which further validate our finding of no association between rs9296559-C and CSF pathologic proteins in CN and AD patients.

Discussion

In this study, we provided the first comprehensive evaluation of the impact of *CD2AP* SNP (rs9296559) on AD CSF pathogenic proteins ($A\beta_{1-42}$, t-tau, and p-tau) in CN, MCI, and AD subjects. Our results showed that CC+TC genotypes were significantly associated with increased CSF t-tau and p-tau levels in MCI groups but did not alter CSF tau levels in AD. Among the existing hypothetical models of dynamic CSF biomarkers in the AD continuum, the most recognized one hypothesizes the sigmoid

Journal Pre-proofs

shape of CSF tau level changes over time, indicating that CSF tau levels increase much slower and even stay the same after MCI patients convert to AD [20]. Several recent longitudinal studies have also revealed the within-individuals reduction of CSF tau levels in AD, which provides more evidence of slowing down the rate of tau-related neuronal injury and dysfunction [21-23]. Combined with the role of CD2AP as a modulator of tau-mediated neurotoxicity, the change of CSF tau trajectory might explain why CD2AP's moderating effect in CSF tau levels is weakened as the disease progresses. Moreover, there was no association between CSF $A\beta_{1-42}$ level and rs9296559 in different disease statuses at baseline. These findings supported that CD2AP modified AD risk by altering tau-induced neurodegeneration in the early stage of the AD continuum.

CD2AP was one of the leading susceptibility genes for SAD, and the possible mechanisms of CD2AP involved in the pathogenesis of SAD have been studied by a number of researchers during the recent years [24-25]. Both Aβ and tau pathologies have been reported to be linked with loss of function of CD2AP. A previous study demonstrated that CD2AP could affect Aβ metabolism in vitro but these effects were subtle in vivo [9]. These results coincided with our results that rs9296559 did not alter CSF $A\beta_{1-42}$ level [7]. Another study showed that CD2AP served as a regulator of endosomal traffics and CD2AP loss of function affects APP and BACE1 sorting in early endosomes, subsequently regulated Aβ generation [8]. More recently, Kotaro et al. found overexpression of CD2AP affected the localization of APP to Rab5-positive early endosomes and Rab7-positive late endosomes, and accelerated the degradation of APP. CD2AP is also related to starvation-induced APP degradation [26].

CD2AP is also linked with another hallmark of AD-neurofibrillary tangles, which is formed by abnormally phosphorylated tau. The fly ortholog of the human

CD2AP, cindr, was reported as a regulator of tau-mediated neurotoxicity. Knockout of cindr (cindr-/-) enhances tau-related neuronal loss in the adult fly brain and reduces the survival time of fly. Moreover, cindr is associated with synapse maturation, synaptic vesicle recycling and release, as well as cytosolic calcium homeostasis [10]. The results of these studies were in line with the present study that modulating tau pathology was an important mechanism for CD2AP to increase the risk of AD.

Conclusion

Collectively, we analyzed the association of *CD2AP* rs9296559 SNP with AD CSF pathogenic proteins for the first time and found that the CC+TC genotype was associated with increased CSF tau levels in MCI, providing further evidence for the hypothesis that CD2AP may contribute to the AD risk by regulating tau pathology. With the limitation of small sample size and different ethnic backgrounds, further studies were needed in the larger samples and even other ethnicities.

Abbreviations

CD2AP: CD2-associated protein； AD: Alzheimer's disease； Aβ: β-amyloid； CN: cognitive normal； MCI: mild cognitive impairment； ADNI: Alzheimer's disease Neuroimaging Initiative； CSF: cerebrospinal fluid； PET: positron emission tomography； SAD: sporadic AD； GWAS: genome-wide association studies； MCI: mild cognitive impairment； DSM-IV：Diagnostic and Statistical Manual of Mental Disease； NIA-AA: National Institute on Aging and Alzheimer's Association； t-tau:total tau； p-tau: phosphorylated tau.

Declarations

Ethics approval and informed consent

The research was approved by the Ethics Committee of the second affiliated hospital of Zhejiang University School of Medicine. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Patients signed informed consent regarding publishing their data.

Data availability

Data will be available upon reasonable request.

Funding

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research and Development, LLC.; Johnson and Johnson Pharmaceutical Research and Development LLC.; Lumosity; Lundbeck; Merck and Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the

Journal Pre-proofs

National Institutes of Health (https://www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. This study was supported by grants from the National Natural Science Foundation of China (81970998), the Key Research and Development project of Zhejiang Province (2019C03039), and the Natural Science Foundation of Zhejiang province (LBY21H090003).

Disclosure

The authors declared no potential conflicts of interest.

CRediT authorship contribution statement

Xue Yanyan: Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **Chen Yihe:** Investigation, Methodology, Visualization. **Lin Rongrong:** Investigation, Methodology, Formal analysis. **Huang Huifen**: Investigation, Visualization. **Wu Zhiying:** Resources, Conceptualization, Funding acquisition, Writing - Review & Editing,. **Tao Qingqing:** Conceptualization, Funding acquisition, Writing - Review & Editing, Supervision.

Acknowledgements

Data used in preparation of this article were obtained from the Alzheimer's disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at:http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

Journal Pre-proofs

References

1. Scheltens P, Blennow K, Breteler MM et al. Alzheimer's disease. *Lancet.* 2016;388:505-517. https://doi.org/10.1016/S0140-6736(15)01124-1

2. Zetterberg H, Bendlin BB. Biomarkers for Alzheimer's disease-preparing for a new era of disease-modifying therapies. *Mol Psychiatry.* 2021;26:296-308. https://doi.org/10.1038/s41380-020-0721-9

3. Jack CR Jr, Bennett DA, Blennow K. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* 2018;14:535-562. https://doi.org/10.1016/j.jalz.2018.02.018

4. Kirsch KH, Georgescu MM, Ishimaru S. CMS: an adapter molecule involved in cytoskeletal rearrangements. *Proc Natl Acad Sci U S A.* 1999;96:6211-6216. https://doi.org/ 10.1073/pnas.96.11.6211

5. Li C, Ruotsalainen V, Tryggvason K et al. CD2AP is expressed with nephrin in developing podocytes and is found widely in mature kidney and elsewhere. *Am J Physiol Renal Physiol.* 2000;279:F785-792. https://doi.org/10.1152/ajprenal.2000.279.4.F785

6. Dustin ML, Olszowy MW, Holdorf AD et al. A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. *Cell.* 1998;94:667-677.

7. Tao QQ, Liu ZJ, Sun YM et al. Decreased gene expression of CD2AP in Chinese patients with sporadic Alzheimer's disease. *Neurobiol Aging.* 2017;56:

212.e5-212.e10. 8. Ubelmann F, Burrinha T, Salavessa L et al. Bin1 and CD2AP polarise the endocytic generation of beta-amyloid. *EMBO Rep.* 2017;18:102-122. https://doi.org/10.1016/s0092-8674(00)81608-6

9. Liao F, Jiang H, Srivatsan S et al. Effects of CD2-associated protein deficiency on amyloid-beta in neuroblastoma cells and in an APP transgenic mouse model. *Mol Neurodegener.* 2015;10:12. https://doi.org/10.1186/s13024-015-0006-y

10. Ojelade SA, Lee TV, Giagtzoglou N et al. cindr, the Drosophila Homolog of the CD2AP Alzheimer's Disease Risk Gene, Is Required for Synaptic Transmission and Proteostasis. *Cell Rep*. 2019;28:1799-1813 e1795. https://doi.org/10.1016/j.celrep.2019.07.041

11. Hollingworth P, Harold D, Sims R et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet.* 2011;43:429-435. https://doi.org/10.1038/ng.803

12. Naj AC, Jun G, Beecham GW et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet.* 2011;43:436-441. https://doi.org/10.1038/ng.801

13. Lambert JC, Ibrahim-Verbaas CA, Harold D et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet.* 2013;45:1452-1458. https://doi.org/10.1038/ng.2802

14. Chen H, Wu G, Jiang Y et al. Analyzing 54,936 Samples Supports the Association Between CD2AP rs9349407 Polymorphism and Alzheimer's Disease Susceptibility. *Mol Neurobiol.* 2015;52:1-7. https://doi.org/10.1007/s12035-014-8834-2

15. Vardarajan BN, Ghani M, Kahn A et al. Rare coding mutations identified by sequencing of Alzheimer disease genome-wide association studies loci. *Ann Neurol.* 2015;78:487-498. https://doi.org/10.1002/ana.24466

16. Kunkle BW, Grenier-Boley B, Sims R et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. *Nat Genet.* 2019;51:414-430. https://doi.org/10.1038/s41588-019-0358-2

17. Prendecki M, Kowalska M, Łagan-Jędrzejczyk U et al. Genetic factors related to the immune system in subjects at risk of developing Alzheimer's disease. *J Integr Neurosci.* 2020;19:359-371. https://doi.org/10.31083/j.jin.2020.02.110

18. Ye LQ, Li XY, Zhang YB et al. The discriminative capacity of CSF beta-amyloid 42 and Tau in neurodegenerative diseases in the Chinese population. *J Neurol Sci.* 2020;412:116756. https://doi.org/10.1016/j.jns.2020.116756

19. Bittner T, Zetterberg H, Teunissen CE et al. Technical performance of a novel, fully automated electrochemiluminescence immunoassay for the quantitation of beta-amyloid (1-42) in human cerebrospinal fluid. *Alzheimers Dement.* 2016;12:517-526. https://doi.org/10.1016/j.jalz.2015.09.009

20. Jack CR Jr, Knopman DS, Jagust WJ et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* 2010;9:119-128. https://doi.org/10.1016/S1474-4422(09)70299-6

21. Hampel H, Buerger K, Kohnken R et al. Tracking of Alzheimer's disease progression with cerebrospinal fluid tau protein phosphorylated at threonine 231. *Ann*

Neurol. 2001;49:545-546

22. Toledo JB, Xie SX, Trojanowski JQ et al. Longitudinal change in CSF Tau and Abeta biomarkers for up to 48 months in ADNI. *Acta Neuropathol.* 2013;126:659-670. https://doi.org/10.1007/s00401-013-1151-4

23. Fagan AM, Xiong C, Jasielec MS et al. Longitudinal change in CSF biomarkers in autosomal-dominant Alzheimer's disease. *Sci Transl Med*. 2014;6(226):226ra30. https://doi.org/10.1126/scitranslmed.3007901

24. Rosenberg RN, Lambracht-Washington D, Yu G. Genomics of Alzheimer Disease: A Review. *JAMA Neurol.* 2016;73:867-874. https://doi.org/10.1001/jamaneurol.2016.0301

25. Tao QQ, Chen YC, Wu ZY. The role of CD2AP in the Pathogenesis of Alzheimer's Disease. *Aging Dis.* 2019;10:901-907. https://doi.org/10.14336/AD.2018.1025

26. Furusawa K, Takasugi T, Chiu YW et al. CD2-associated protein (CD2AP) overexpression accelerates amyloid precursor protein (APP) transfer from early endosomes to the lysosomal degradation pathway. *J Biol Chem.* 2019;294:10886-10899. https://doi.org/10.1074/jbc.RA118.005385

Journal Pre-proofs

Legends of Figures

Fig.1 Data analysis workflow.

Fig.2 Scatterplots of CSF $A\beta_{1-42}$, p-tau, and t-tau levels in CN, MCI and AD subjects from ADNI. Mean CSF $A\beta_{1-42}$, p-tau, and t-tau levels were higher in MCI and AD subjects compared with CN subjects ($P < 0.0001$)

Fig.3 *CD2AP* rs9296559 and CSF $A\beta_{1-42}$, p-tau, and t-tau levels at baseline in the CN, MCI and AD subjects from ADNI. **(A)** No significant differences of CSF $A\beta_{1-42}$, p-tau, and t-tau levels were observed between subjects carrying TT and CC+TC in CN group. **(B)** No significant differences of $A\beta_{1-42}$ level between subjects carrying TT and CC+TC in MCI group. P-tau and t-tau levels were significant elevated in subjects carrying CC+TC in MCI group ($P < 0.05$). **(C)** No significant differences of CSF $A\beta_{1-42}$, p-tau, and t-tau levels were observed between subjects carrying TT and CC+TC in AD group.

Fig.4 Scatterplots of CSF $A\beta_{1-42}$, p-tau, and t-tau levels in CN, MCI and AD subjects in an independent cohort.

Journal Pre-proofs

Fig.5 *CD2AP* rs9296559 and CSF $A\beta_{1-42}$, p-tau, and t-tau levels at baseline in the CN, MCI and AD subjects in an independent cohort. **(A)** No significant differences of CSF $A\beta_{1-42}$, p-tau, and t-tau levels were observed between subjects carrying TT and CC+TC in CN group. **(B)** No significant differences of $A\beta_{1-42}$ level between subjects carrying TT and CC+TC in MCI group. In the MCI group, p-tau level was significant elevated in subjects carrying CC+TC ($P < 0.05$), but t-tau level was insignificantly increased in subjects carrying CC+TC ($P=0.06$). **(C)** No significant differences of CSF $A\beta_{1-42}$, p-tau, and t-tau levels were observed between subjects carrying TT and CC+TC in AD group.

Journal Pre-proofs

Table 1. Demographics and characteristics of the study participants from the ADNI cohort

Characteristics	**Total (N=543)**	**CN (N=101)**	**MCI (N=278)**	**AD (N=164)**	***P*** [a]	***P*** [b]	***P*** [c]
Age, mean years (SD)	74.01±7.36	75.38±5.35	73.09±7.70	74.74±7.65	0.084	>0.999	0.055
Gender (male/female)	324/219	54/47	175/103	95/69	0.095	0.477	0.295
Education, mean years (SD)	15.87±2.87	15.89±2.73	16.09±2.93	15.49±2.82	>0.999	0.797	0.066
APOE ε4 (0/1/2)	246/221/76	76/23/2	117/122/39	53/76/35	<0.0001	<0.0001	0.05
MMSE scores, mean (SD)	26.49±2.78	29.15±1.01	27.35±1.85	23.37±1.98	<0.0001	<0.0001	<0.0001
CD2AP rs9296559, T/C	770/316	159/43	380/176	231/97	<0.01	<0.05	0.518
CSF $A\beta_{1\text{-}42}$ (pg/ml), mean (SD)	904.91±544.26	1243.99±652.63	913.86±507.38	680.92±405.40	<0.0001	<0.0001	<0.0001
CSF t-tau (pg/ml), mean (SD)	312.58±141.88	238.40±86.29	308.24±142.70	365.63±146.59	<0.0001	<0.0001	<0.0001
CSF p-tau (pg/ml), mean (SD)	30.62±15.56	22.11±9.03	30.28±15.74	36.42±15.96	<0.0001	<0.0001	<0.0001

Data are given as mean followed by (SD).

P [a] values when comparing CN and MCI groups.

P [b] values when comparing CN and AD groups.

P [c] values when comparing MCI and AD groups.

Abbreviation: CN, cognitive normal; MCI, mild cognitive impairment; MMSE, mini-mental state exam; SD, standard deviation.

Journal Pre-proofs

Table 2. Correlations between *CD2AP* rs9296559 and CSF biomarkers in CN, MCI and AD subjects

Biomarker	CN		MCI		AD	
	β coefficient	*P*	β coefficient	*P*	β coefficient	*P*
CSF $A\beta_{1-42}$	-0.001	0.988	0.012	0.819	-0.104	0.150
CSF t-tau	-0.095	0.337	**0.144**	**0.013**	0.011	0.891
CSF p-tau	-0.091	0.353	**0.137**	**0.018**	0.024	0.756

Abbreviation: CN, cognitive normal; MCI, mild cognitive impairment; AD, Alzheimer's disease.

Table 3. Demographics and characteristics of the study participants in an independent cohort

Characteristics	**Total (N=198)**	**CN (N=55)**	**MCI (N=33)**
Age, mean years (SD)	62.24±9.26	57.98±13.25	62.03±10.94
Gender (male/female)	109/89	37/18	16/17
Education, mean years (SD)	7.87±4.13	8.67±4.31	7.82±3.94
APOE ε4 (0/1/2)	125/59/14	39/14/2	20/8/5
CD2AP rs9296559, T/C	332/64	88/22	60/6
CSF $A\beta_{1-42}$ (pg/ml), mean (SD)	704.90±359.97	989.79±261.79	745.68±374.52
CSF t-tau (pg/ml), mean (SD)	329.32±274.71	98.27±49.39	239.92±173.86
CSF p-tau (pg/ml), mean (SD)	57.64±38.25	21.49±9.47	49.68±23.04

Data are given as mean followed by (SD).

Abbreviation: CN, cognitive normal; MCI, mild cognitive impairment; MMSE,

Journal Pre-proofs

mini-mental state exam; SD, standard deviation.

- There was no association between CSF $A\beta_{1-42}$ level and *CD2AP* rs9296559 SNP in CN, MCI or AD
- CC+TC genotype of rs9296559 was associated with increased CSF tau levels in MCI
- CC+TC genotype of rs9296559 did not alter CSF tau levels in CN or AD.

Journal Pre-proofs