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ORIGINAL ARTICLE

# A Genome-Wide Association Study of α-Synuclein Levels in Cerebrospinal Fluid

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## Abstract

α-Synuclein is a 140-amino acid protein produced predominantly by neurons in the brain which plays a role in the regulation of neurotransmitter release, synaptic function, and plasticity, thus making it the focus in understanding the etiology of a group of neurodegenerative diseases. We conducted genome-wide association studies (GWAS) of α-synuclein levels in cerebrospinal fluid (CSF) with 209 non-Hispanic white participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI-1) cohort using a linear regression model to identify novel variants associated with α-synuclein concentration. The minor allele (T) of rs7072338 in the long intergenic non-protein coding RNA 1515 (LINC01515) and the minor allele (T) of rs17794023 in clusterin-associated protein 1 (CLUAP1) were associated with higher CSF α-synuclein levels at genome-wide significance ($P = 4.167 \times 10^{-9}$ and $9.56 \times 10^{-9}$, respectively). In addition, single nucleotide polymorphisms (SNPs) near amyloid beta precursor protein (APP) (rs1394839) ($P = 2.31 \times 10^{-7}$), Rap guanine nucleotide exchange factor 1 (RAPGEF1) (rs10901091) ($P = 8.07 \times 10^{-7}$), and two intergenic loci on chromosome 2 and 14 (rs11687064 $P = 2.50 \times 10^{-7}$and rs7147386 $P = 4.05 \times 10^{-7}$) were identified as suggestive loci associated with CSF α-synuclein levels. We have identified significantly associated SNPs for CSF α-synuclein. These associations have important implications for a better understanding of α-synuclein regulation and allow researchers to further explore the relationships between these SNPs and α-synuclein-related neurodegenerative disorders.

**Keywords** α-Synuclein · Cerebrospinal fluid · Endophenotype · Genome-wide association studies

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (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.

Xiao-ling Zhong and Jie-Qiong Li contributed equally to this work and should be considered co-first authors.

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## Introduction

From the time of its discovery, α-synuclein, a 140-amino acid protein produced predominantly by neurons in the brain, has been the focus in understanding the etiology of a group of neurodegenerative diseases called α-synucleinopathies. These include Parkinson's disease (PD), dementia with Lewy bodies (DLB) (Spillantini et al. 1997) and multiple system atrophy (MSA) (Gai et al. 1998). Moreover, α-synuclein regulates the fibrilization of both amyloid-β (Aβ) and tau, two key proteins in Alzheimer's disease (AD) pathophysiology (Bachhuber et al. 2015; Giasson et al. 2003; Guo et al. 2013; Masliah et al. 2001; Yoshimoto et al. 1995), which suggests an important role for α-synuclein toxicity in neurodegeneration. The quantification of α-synuclein in CSF is in parallel with the measurement of proteins in CSF related to AD, namely total and phosphorylated tau protein and β-amyloid. Therefore, α-synuclein has gained much attention as a potential biomarker of α-synuclein-related neurodegenerative disorders in recent years. α-Synuclein was thought at first to be an exclusively intracellular protein and this notion was challenged when α-synuclein was detected in biological fluids, such as CSF (El-Agnaf et al. 2003; Mollenhauer et al. 2008). A number of studies have evaluated the potential of CSF α-synuclein as a diagnostic biomarker for α-synucleinopathies, but the results were inconsistent (Hong et al. 2010; Korff et al. 2013; Mollenhauer et al. 2008, 2010; Shi et al. 2011; Toledo et al. 2013; Wang et al. 2012). In general, patients with synucleinopathies, e.g., PD, DLB, and MSA often have reduced CSF α-synuclein compared to controls, while in AD patients, CSF α-synuclein levels were often higher as compared with cognitively healthy controls. Although the normal function of α-synuclein remains unclear, studies suggest that α-synuclein has a role in the regulation of neurotransmitter release, synaptic function, and plasticity (Lashuel et al. 2013). A pathological role for α-synuclein in these diseases is further supported by various genetic evidences. Multiplication of the gene encoding α-synuclein (SNCA) and six missense mutations ((A30P, E46K, H50Q, G51D, A53E, and A53T) in this gene are identified to be associated with dominant familial Parkinsonism (Appel-Cresswell et al. 2013; Kruger et al. 1998; Lesage et al. 2013; Pasanen et al. 2014; Polymeropoulos et al. 1997; Proukakis et al. 2013; Zarranz et al. 2004). In addition, multiple genome-wide association studies (GWAS) have identified SNPs in SNCA as major risk factors for sporadic PD (Simon-Sanchez et al. 2009). Nevertheless, the molecular mechanisms by which α-synuclein aggregation contributes to neurodegeneration remain unclear.

The use of quantitative traits in GWAS has been shown to increase statistical power over case-control designs (Cruchaga et al. 2013; Kim et al. 2011). Here, on the basis of adequate evidence on the role of CSF α-synuclein in neurodegenerative disorders, we conducted a GWAS of CSF from ADNI database. Further examinations of the variants that we have identified in different datasets may lead to a deeper understanding of α-synuclein regulation and provide important insights into its effects on α-synuclein-related function and disorders.

## Methods

### ADNI Study Design

Data used in this study were obtained from the ADNI database (http://adni.loni.usc.edu). The most recent information from the ADNI is available online (http://www.adni-info.org). The ADNI is a large, multicenter, longitudinal neuroimaging study, launched 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. The study gathered and analyzed thousands of brain scans, genetic profiles, and biomarkers in blood and cerebrospinal fluid. This study was approved by institutional review boards of all participating institutions and written informed consent was obtained from all participants or authorized representatives.

### Participants and CSF Measurement

Our study population consisted of all CN, MCI, and AD dementia group participants from the ADNI-1. In this study, 686 (CN = 194, MCI = 330, AD = 160 at baseline) non-Hispanic Caucasian individuals from the ADNI cohort whose data met all quality control criteria were included, which would reduce the likelihood of population stratification effects in the GWAS.

CSF samples were collected from individuals in the Alzheimer's Disease Neuroimaging Initiative (ADNI). Levels of CSF α-synuclein concentration were measured using LuminexMicroPlex (Luminex Corp, Austin, TX). The α-synuclein Luminex assay demonstrated low day-to-day as well as plate-to-plate signal variability. The accuracy for the assay, as determined by recovery of spiked α-synuclein, was ~ 93%(Toledo et al. 2013).

### Genotyping and Quality Control in GWAS

The ADNI samples were genotyped with the Illumina 610 chip. Given the smaller size of the current sample as compared to previous analyses, several quality control measures were applied to the 620,901 SNPs to detect potential biases in genotyping using the PLINK software package. Only SNPs with a minor allele frequency (MAF) > 5%, call rates > 98%, and Hardy-Weinberg equilibrium $P > 0.001$ were retained for analysis. Finally, a maximum of 519,442 SNPs were retained after these procedures. On the basis of data for all of these SNPs, we excluded 151 individuals who had more than 5% missing genotypes within 757 samples. This more stringent threshold was chosen to reduce the likelihood of false-positive results in the context of modest sample

size. In order to decrease CSF contamination by RBC, a human hemoglobin ELISA quantitation kit was used (https://ida.loni.usc.edu/pages/access/studyData.jsp), which has sensitivity well beyond the cut-off value of 1000 ng/ml (Hall et al. 2012). For this reason, 326 samples were removed. In addition, we excluded 71 samples that were non-Hispanic Caucasians. Finally, 209 individuals with CSF α-synuclein were retained at last.

### Statistical Analyses

The distribution of α-synuclein levels were approximately considered as normal distribution after log transformation. One-way ANOVA models were used for quantitative normally distributed variables. Rank-based two-way methods were used for non-normally distributed quantitative variables (Toledo et al. 2013). Chi-square text was applied to categorical data (Wang et al. 2016). To examine the main effect of each SNP on the CSF α-synuclein biomarker, GWAS was performed with additive genetic model. We used a multiple linear regression model to estimate possible correlation between genotypes and CSF α-synuclein concentration (e.g., dose-dependent effect of the minor allele). Covariates such as age, gender, *APOE* ε4 status, educational level, and baseline disease status were considered and retained in the final models if $P < 0.05$. We focused on SNPs with uncorrected $P < 5 \times 10^{-8}$ (or Bonferroni correction correct $P < 0.01$) as genome-wide significant and secondarily examined SNPs with $P$ values less than $1 \times 10^{-5}$ to identify potential candidates (Risch and Merikangas 1996). All statistical analyses were performed by R 3.4.0 and PLINK (http://pngu.mgh.harvard.edu/wpurcell/plink/).

## Results

### Demographic Characteristic and CSF α-Synuclein Concentration

The detailed demographics of 209 (CN = 59, MCI = 101, AD = 49 at baseline) non-Hispanic Caucasian participants at baseline diagnosis were summarized in Table 1. No difference was found across the diagnostic groups for age, education, and sex ($P > 0.05$). Compared to CN and MCI subjects, AD individuals have higher CSF α-synuclein concentration, higher frequency of *APOE* ε4 allele, and worst cognitive function displayed by neuropsychological scales (MMSE and CDR-SB) ($P < 0.05$). In addition, associations were detected between baseline demographics (e.g., *APOE* ε4 status, disease status, and educational years) and CSF α-synuclein level ($P < 0.05$), which were considered as the evidence of covariates.

### Loci Associated with CSF α-Synuclein Levels

Relationships between 519,442 SNPs and CSF α-synuclein levels were shown in a Manhattan plot, with *APOE* ε4 status, disease status, and educational years included as covariates (Fig. 1). The obtained genomic inflation factors of CSF biomarker associations ($\lambda = 1.00$) indicated a low risk of confounding due to population stratification. Six SNPs in the regions of long intergenic non-protein coding RNA 1515 (LINC01515) and clusterin-associated protein 1 (CLUAP1) reached genome-wide significance (unadjusted $P < 10^{-7}$, adjusted $P < 0.01$). In addition, SNPs near APP (rs1394839) ($P = 2.31 \times 10^{-7}$), RAPGEF1 (rs10901091) ($P = 8.07 \times 10^{-7}$), and two intergenic loci on chromosome 2 and 14 (rs11687064 $P = 2.50 \times 10^{-7}$ and rs7147386 $P = 4.05 \times 10^{-7}$) were identified as suggestive loci associated with CSF α-synuclein levels (Table 2). All the annotation information of SNPs that did not reach genome-wide significance or uncorrected $P$ values less than $10^{-5}$ were listed in Supplementary Table 1.

Among all the SNPs, rs7072338, which is located in the intron region of LINC01515 on chromosome 10, showed the strongest association with CSF α-synuclein (uncorrected $P = 4.167 \times 10^{-9}$, Bonferroni corrected $P = 2.164 \times 10^{-3}$). Another four SNPs located near rs7072338 also reached a GWAS significant $P$ value (uncorrected $P = 1.909 \times 10^{-8}$, Bonferroni corrected $P = 9.917 \times 10^{-3}$). Besides that, three SNPs around rs7072338 showed a $P$ value lower than $10^{-5}$(Supplementary Table 1). We confirmed the most significant SNP in this locus and other seven SNPs in linkage disequilibrium (LD, $r^2 > 0.8$)

**Table 1** Demographic information of cohort for GWAS

| | CN ($n = 59$) | MCI ($n = 101$) | AD ($n = 49$) | $P$ value |
|---|---|---|---|---|
| Age (mean ± SD) | 76.12 ± 4.64 | 74.23 ± 7.63 | 74.16 ± 8.63 | 0.384 |
| Education (mean ± SD) | 16.32 ± 2.47 | 15.69 ± 3.16 | 15.12 ± 3.33 | 0.2924 |
| Sex (male/female) | 32/27 | 68/33 | 23/26 | 0.1022 |
| MMSE | 29.24 ± 0.86 | 26.83 ± 1.74 | 23.59 ± 1.84 | < 0.01 |
| CDR-SB | 0.02 ± 0.09 | 1.58 ± 0.84 | 4.37 ± 1.43 | < 0.01 |
| *APOE* ε4 (0/1/2) | 45/12/2 | 44/47/10 | 17/20/12 | < 0.01 |
| α-Synuclein (ng/ml) (mean ± SD) | 0.50 ± 0.33 | 0.52 ± 0.20 | 0.65 ± 0.45 | < 0.01 |

*P* values are from the Kruskal-Wallis test or Fisher exact test

*AD* Alzheimer disease, *MCI* mild cognitive impairment, *CN* cognition normal, *ALL* total subjects, *MMSE* Mini-Mental State Exam, CDR-SB Clinical Dementia Rating Sum of Boxes

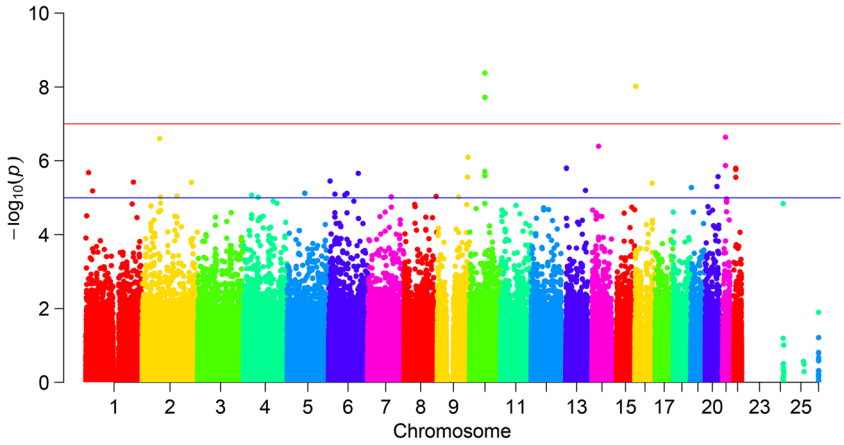


**Fig. 1** Manhattan plot for the GWAS of CSF α-synuclein biomark. Observed $-\log_{10} P$ values (*y*-axis) are displayed for all tested SNPs on each autosomal chromosome (*x*-axis). The red horizontal line at $10^{-7}$ indicates genome-wide significance

(Fig. 2a). However, after controlling for rs7072338 genotype, no SNPs in this region showed an association with CSF α-synuclein levels indicating that all the association in this locus was driven by rs7072338 (Fig. 2b). In addition, the linkage disequilibrium (LD) pattern between rs7072338 and nearby SNPs was almost identical in the ADNI cohort compared with 1000 Genomes European subjects (Supplementary Fig. 1), suggesting that the SNP genotypes from this study were accurate. The minor allele (T) of rs7072338 was associated with higher CSF α-synuclein levels in a dose-dependent effect within both combined groups and each diagnostic group (normal group, $p = 5.14 \times 10^{-5}$; pMCI group, $p = 3.77 \times 10^{-3}$; sMCI group, $P = 0.034$ and AD group, $P = 8.56 \times 10^{-4}$) (Supplementary Fig. 2).

Moreover, rs17794023, located in CLUAP1, also showed a genome-wide significant association with CSF α-synuclein levels ($P = 9.56 \times 10^{-9}$). This locus survived even after Bonferroni corrections for multiple testing (Bonferroni corrected $P = 4.964 \times 10^{-3}$). The minor allele (T) of rs17794023 was associated with higher CSF α-synuclein levels in a dose-dependent effect within both combined groups and each diagnostic group (normal group, $P = 2.81 \times 10^{-3}$; pMCI group, $P = 3.32 \times 10^{-3}$; sMCI group, $p = 0.56$ and AD group, $p = 3.14 \times 10^{-5}$) (Supplementary Fig. 3).

## Discussion

To our knowledge, we firstly performed a GWAS of CSF α-synuclein levels in the ADNI cohort. Six SNPs in the regions of LINC01515 and CLUAP1 were identified to be genome-wide significant loci associated with CSF α-synuclein levels. Among them, the significant association of SNPs in LINC01515 was driven by rs7072338. Ultimately, we detected two SNPs (LINC01515 (rs7072338) and CLUAP1 (rs17794023)) are associated with CSF α-synuclein levels. Moreover, SNPs near APP (rs1394839), RAPGEF1 (rs10901091), and two intergenic loci on chromosome 2 and 14 (rs11687064 and rs7147386) were identified as suggestive loci associated with CSF α-synuclein levels. Function of LINC01515 has never been explored yet, while CLUAP1

**Table 2** Association results for CSF α-synuclein in ADNI

| CHR | SNP | BP | A1 | MAF | *Closest.Gene* | SNP.Type | BETA | *P* |
|---|---|---|---|---|---|---|---|---|
| 10 | rs7072338 | 67,188,144 | T | 0.06516 | *LINC01515* | Intron variant | 0.4461 | $4.17 \times 10^{-9}$ |
| 16 | rs17794023 | 3,524,180 | T | 0.0754 | *CLUAP1* | Intron variant | 0.3193 | $9.56 \times 10^{-9}$ |
| 10 | rs10762004 | 67,149,453 | T | 0.05812 | *LINC01515* | Intron variant | 0.4406 | $1.91 \times 10^{-8}$ |
| 10 | rs10996614 | 67,158,548 | C | 0.05284 | *LINC01515* | Intron variant | 0.4406 | $1.91 \times 10^{-8}$ |
| 10 | rs7085632 | 67,166,307 | G | 0.06011 | *LINC01515* | Intron variant | 0.4406 | $1.91 \times 10^{-8}$ |
| 10 | rs7086079 | 67,166,620 | G | 0.05232 | *LINC01515* | Intron variant | 0.4406 | $1.91 \times 10^{-8}$ |
| 21 | rs1394839 | 26,136,162 | G | 0.1691 | *APP* | − 38,570 bp | 0.2238 | $2.31 \times 10^{-7}$ |
| 2 | rs11687064 | 76,272,974 | G | 0.08124 | *Unknown* | Intergenic | 0.3049 | $2.50 \times 10^{-7}$ |
| 14 | rs7147386 | 47,938,231 | A | 0.06275 | *Unknown* | Intergenic | 0.325 | $4.05 \times 10^{-7}$ |
| 9 | rs10901091 | 133,634,029 | G | 0.06473 | *RAPGEF1* | + 28,747 bp | 0.2749 | $8.07 \times 10^{-7}$ |

*CHR* chromosome, *SNP* single nucleotide polymorphism, *BP* base pair location in release 19, build 135 of the human genome in the dbSNP database, *A1* the minor allele, *MAF* minor allele frequency in ADNI, *SNP.Type* type of SNP, *BETA* change CSF α-synuclein per copy of the minor allele, in which positive numbers indicate more rapid decline and negative numbers indicate slower decline, *P* relationship between SNPs and CSF α-synuclein using multiple linear regression model adjusted for educational years, *APOE* ε4 status, and baseline disease status

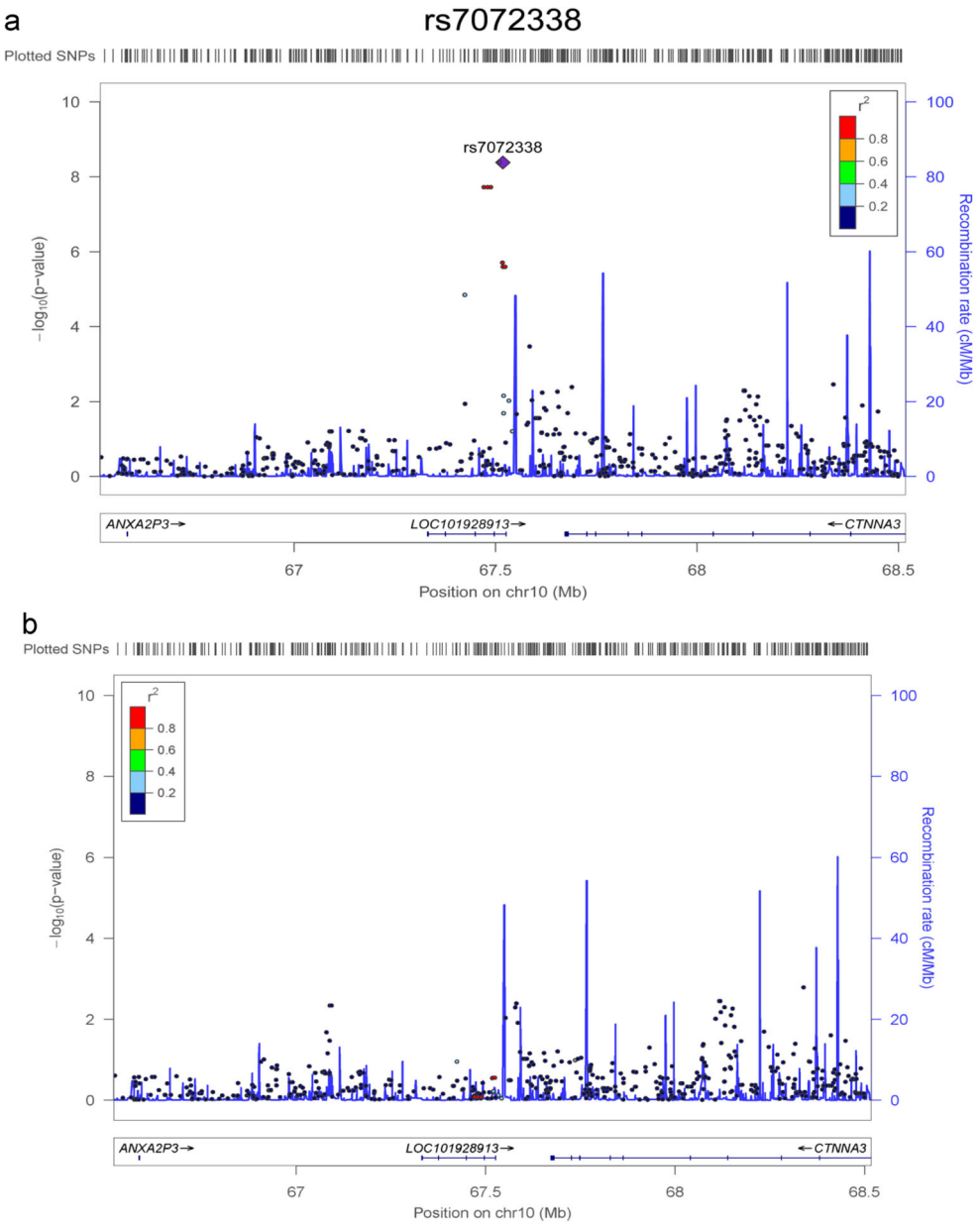


**Fig. 2** **a** Regional association results for the LINC01515 region of chromosome 10 Fig. **b** Association results for 10q21.3 controlling for rs7072338

appears to be involved in AD-linked cognitive deterioration as a consequence of their interactions with Aβs (Armato et al. 2013). Previous studies indicated that CLUAP1 is involved in ciliogenesis and impacts cognitive deterioration in AD as a consequence of the neurogenesis process occurring in the hippocampus (Armato et al. 2013; Botilde et al. 2013). Besides causing cognition impairment, missense mutation in the CLUAP1 gene was also found to modify the age of onset in PSEN1 E280A AD (Velez et al. 2016). Beyond that, CLUAP1 plays an important role in carcinogenesis of multiple types of tumors such as osteosarcomas, ovarian, colon, and lung cancers and may be useful as a tumor-associated antigen or a novel therapeutic intervention for treatment in multiple malignancies (Ishikura et al. 2007; Takahashi et al. 2004). Interestingly, our analysis identified one SNP near APP gene as a suggestive locus. Mutations in APP that increase production of APP-derived Aβ cause autosomal dominant forms of familial AD (FAD) (Selkoe 2001). Aβ plaques and α-synuclein-rich Lewy bodies are the major neuropathological hallmarks of Alzheimer's disease (AD) and Parkinson's disease. Evidence from animal models shows that Aβ may contribute to the development of Lewy body diseases by promoting the aggregation of α-synuclein and exacerbating α-synuclein-dependent neuronal pathologies (Masliah et al. 2001). In addition, α-synuclein may lead to inhibition of Aβ deposition and reduced plaque formation (Bachhuber et al. 2015). The relationships between Aβ and α-synuclein still need further research.

Rs7072338 and rs17794023 are intronic SNPs which can affect protein structure by regulation of alternative splicing,

positive regulation of gene expression, and regulation of nonsense-mediated decay (Jo and Choi 2015), and have even been experimentally shown to affect transcription (Greenwood and Kelsoe 2003). Therefore, they may play an important role in α-synuclein levels. In fact, most of the SNPs detected by traditional case-control GWASs have been mapped to intron regions rather than exonic or nonsynonymous sites (Li et al. 2012; Welter et al. 2014). Investigation of the functional implication of these intronic SNPs will thus be an important research subject in the future. However, our data are not whole exome or genome and full sequencing data within the region may reveal other candidate causal variants. Further exploration in larger populations will be necessary to assess whether and how these SNPs contribute to α-synuclein-related functions and disorders. In addition, participants included in our study were AD oriented; according to the results of subgroup analysis, our findings could be generalized to cognitively normal population. However, whether these findings could be generalized to other populations (e.g., Parkinson disease) has never been assessed and still needs further exploration.

## Conclusion

We have identified an association between two genetic significant variants and four suggestive loci with CSF α-synuclein levels. Our results have important implications for a better understanding of α-synuclein regulation and allow researchers to further explore the relationships between these SNPs and α-synuclein-related neurodegenerative disorders.

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## Compliance with Ethical Standards

The study procedures were approved by the institutional review boards of all participating centers (https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf). Ethics approval was obtained from the institutional review boards of each institution involved: Oregon Health and Science University; University of Southern California; University of California—San Diego; University of Michigan; Mayo Clinic, Rochester; Baylor College of Medicine; Columbia University Medical Center; Washington University, St. Louis; University of Alabama at Birmingham; Mount Sinai School of Medicine; Rush University Medical Center; Wien Center; Johns Hopkins University; New York University; Duke University Medical Center; University of Pennsylvania; University of Kentucky; University of Pittsburgh; University of Rochester Medical Center; University of California, Irvine; University of Texas Southwestern Medical School; Emory University; University of Kansas, Medical Center; University of California, Los Angeles; Mayo Clinic, Jacksonville; Indiana University; Yale University School of Medicine; McGill University, Montreal-Jewish General Hospital; Sunnybrook Health Sciences, Ontario; U.B.C.Clinic for AD & Related Disorders; Cognitive Neurology—St. Joseph's, Ontario; Cleveland Clinic Lou Ruvo Center for Brain Health; Northwestern University; Premiere Research Inst (Palm Beach Neurology); Georgetown University Medical Center; Brigham and Women's Hospital; Stanford University; Banner Sun Health Research Institute; Boston University; Howard University; Case Western Reserve University; University of California, Davis—Sacramento; Neurological Care of CNY; Parkwood Hospital; University of Wisconsin; University of California, Irvine—BIC; Banner Alzheimer's Institute; Dent Neurologic Institute; Ohio State University; Albany Medical College; Hartford Hospital, Olin Neuropsychiatry Research Center; Dartmouth-Hitchcock Medical Center; Wake Forest University Health Sciences; Rhode Island Hospital; Butler Hospital; UC San Francisco; Medical University South Carolina; St. Joseph's Health Care Nathan Kline Institute; University of Iowa College of Medicine; Cornell University; and University of South Florida: USF Health Byrd Alzheimer's Institute.

**Conflict of Interest** The authors declare that they have no conflicts of interest.

**Informed Consent** Informed consent was obtained from all participants or their authorized representatives.

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