







RESEARCH ARTICLE

CYLD variants identified in Alzheimer's disease and frontotemporal dementia patients

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Introduction

Dementia is a devastating disease and imposes a huge burden on patients and their caregivers. Currently, approximately 50 million people suffer from dementia and its prevalence is projected to triple by 2050.¹ Alzheimer's disease (AD) is the most prevalent dementia and accounts for 60%–80% of dementia cases.² Frontotemporal dementia (FTD) is the second most common dementia with a prevalence ranging from 3% to 26% in all

Abstract

Objectives: *CYLD* was a novel causative gene for frontotemporal dementia (FTD) and amyotrophic lateral sclerosis. Given the clinical and pathological overlap of FTD and Alzheimer's disease (AD), it is necessary to screen *CYLD* in AD patients and FTD patients in the Chinese population. **Methods:** In our study, using a targeted sequencing panel, we sequenced the *CYLD* gene in a large cohort of 2485 participants in the Chinese population, including 1008 AD patients, 105 FTD patients, and 1372 controls. **Results:** In the present study, the average onset age of AD and FTD patients was 66.84 ± 30.42 years old and 60 ± 10.00 years old, respectively. Our study reported three novel *CYLD* variants: p.Phe288Leu (patient No. 1, AD), p.Tyr485Phe (patients No. 6–9, all AD) and p.Thr951Ala (patient No. 10, AD), plus a previously reported variant: p.Arg397Ser (patient No. 2–5, AD and No. 11, FTD). These variants were absent in our in-house controls and predicted to be deleterious according to the MutationTaster. The variant carriers were composed of 10 AD patients and one FTD patient, and the average onset age was 61.2 ± 10.9 years. The frequency of *CYLD* variants in AD was similar to that in FTD, which was 0.99% (10/1008) and 0.95% (1/105), respectively. **Interpretation:** Our finding extended the genotype and phenotype of the *CYLD* gene and demonstrated that *CYLD* rare damaging variants may be implicated in AD and FTD pathogenesis.

dementia patients.³ Clinically, both AD and FTD exhibit progressive impairments in different cognitive domains, including memory, language, behavior, and execution. Of note, atypical AD cases closely resemble FTD patients.⁴ Aggregation of transactive response (TAR)-DNA binding protein 43 (TDP-43) and tau protein are shared pathological hallmarks in AD and FTD.⁵ Additionally, FTD causative genes identified previously, such as *MAPT*, *C9orf72*, *GRN*, *TARDBP*, *UBQLN1*, *HNRNPA1*, and *CHCHD10* accounted for a proportion of AD pathogenesis.^{6–10} For

example, abnormal *C9orf72* expansions were detected in AD patients but absent from controls.

Dobson-Stone et al. recently identified that *CYLD* was a novel causative gene for frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). A missense variant, *CYLD* p.M719V mutation was segregated in a large family with FTD and ALS. Functional studies revealed that *CYLD* p.M719V was associated with increased TAR DNA-binding protein 43 (TDP-43) and decreased axonal length. Additionally, this variant was involved in the inhibition of nuclear factor-kappa B (NF- κ B) and impaired autophagy.¹¹ Intriguingly, one of the most dominant symptoms in patients with *CYLD* variant is memory impairment and some were clinically diagnosed with Alzheimer's disease (AD).¹² Also, two rare *CYLD* variants were observed in two patients with marked memory loss in a Portuguese cohort.¹³

Given the clinical and pathological overlap between AD and FTD, we hypothesized that *CYLD* may contribute to AD pathogenesis. Besides, to date, no studies have investigated the association of *CYLD* variants AD or FTD in the non-Caucasian population. Consequently, it is necessary to screen *CYLD* variants in AD and FTD patients in the Chinese population. In this study, we sequenced the *CYLD* gene in a large cohort of 2485 individuals using targeted gene sequencing.

Methods

Subjects

Our study recruited 1008 AD patients and 105 FTD patients from Xiangya Hospital and 1372 cognitively normal controls from a community in Changsha. According to the National Institute on Aging-Alzheimer's Association criteria for probable AD and diagnostic criteria for FTD,^{14–15} patients were diagnosed with AD or FTD by two expert neurologists. Patients with causative mutations for AD and FTD, including *APP*, *PSEN1*, *PSEN2*, *MAPT*, *GRN*, *C9orf72*, *CHCHD10*, *CHMP2B*, *VCP*, *TARDBP*, *TBK1*, and *FUS* had been excluded by Targeted genes sequencing or repeat-prime PCR (RP-PCR) analysis. Other inclusion criteria for AD or FTD patients include (1) the disease duration is larger than 6 months; (2) neuropsychological questionnaires indicated the patient had dementia; (3) The modified Hachinski ischemia scale score was ≤ 4 ; (4) The patients or their legal guardians agreed to participate in this study. Additionally, the inclusion criteria for controls involved (1) no chief complaint with cognitive impairment; (2) neuropsychological questionnaires revealed no dementia; (3) they had no other neurological diseases. This study was approved by the Ethics Committee of Xiangya Hospital, Central South University, China.

Genomic DNA isolation

We extracted genomic DNA from the peripheral blood leukocytes via phenol-chloroform extraction and ethanol precipitation.¹⁶ The DNA's quality and quantity were assessed using a NanoDrop spectrophotometer (Thermo Scientific). The DNA sample was diluted to 50–100 ng/ μ L.

Targeted gene sequencing

The targeted sequencing panel comprised *CYLD* and *APOE* genes. The genomic DNA was broken into 150–200 bp length fragments using Biorupter Pico. End-repairing, A-tailing, adaptor ligation, and pre-capture PCR amplification were performed. The DNA was captured by a targeted panel and sequenced on Illumina NovaSeq 6000 platform. Using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), the low-quality reads fastq data were removed. The reads were aligned to the human reference genome (hg19) via the BWA software (<http://bio-bwa.sourceforge.net>).¹⁷ Duplicate sequence reads were discarded with Picard (version 2.18.7, <http://broadinstitute.github.io/picard/>). Using the Genome Analysis Toolkit (version 3.2),¹⁸ we performed the quality-score recalibration, local realignments, and variant calling. Variants were annotated with ANNOVAR (<https://hpc.nih.gov/apps/ANNOVAR.html>).¹⁹ According to minor allele frequencies (MAF), rare variants were defined by variants with MAF < 0.001. Furthermore, the pathogenicity of missense variants was predicted using MutationTaster (<https://www.mutationtaster.org/>), SIFT, and PolyPhen2.

Results

In the present study, the average onset age of AD and FTD patients was 66.84 ± 30.42 years old and 60 ± 10.00 years old, respectively. Besides, the average enrolled age was 67.98 ± 6.75 years old in controls. We identified three *CYLD* novel rare variants, including c.864 T > A, p.Phe288Leu, c.1454A > T, p.Tyr485Phe, and c.2851A > G, p.Thr951Ala, as well as one previously reported variant c.1189C > A:p.Arg397Ser (Fig. 1).²⁰ All these rare *CYLD* variants were verified by Sanger sequencing. These variants exhibited extremely low frequency (minor allele frequency < 0.001) in the Genome Aggregation Database (gnomAD) database and were predicted to be damaging using the MutationTaster (<https://www.mutationtaster.org/>). All these variants were absent in our in-house controls. The variant carriers consisted of 10 AD patients (patients No.1-No.10) and one FTD patient (patient No.11), who had an average onset age of

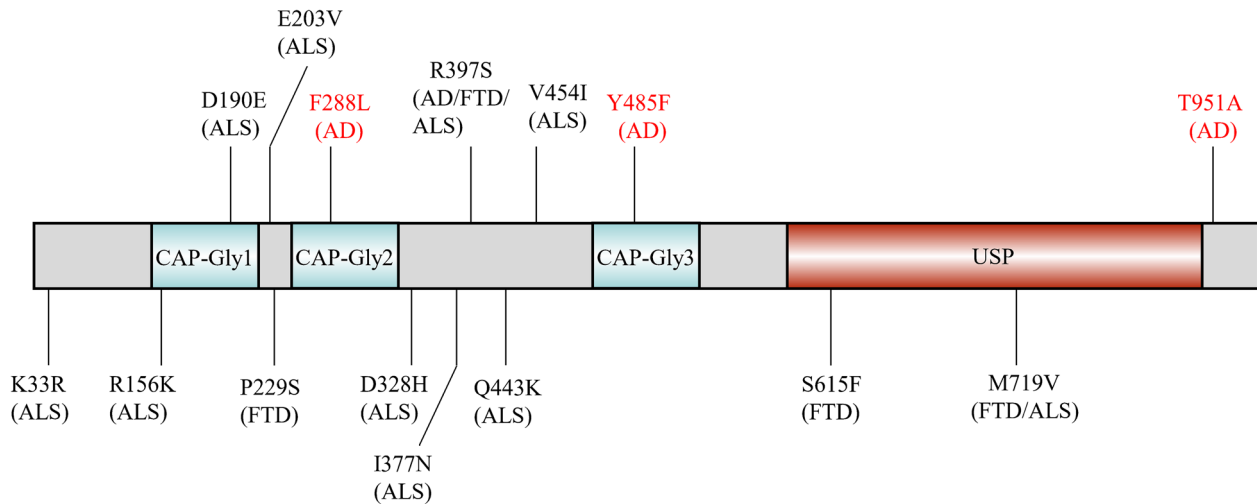


Figure 1. Schematic of rare damaging variants in the *CYLD* gene. AD, Alzheimer's disease; FTD, Frontotemporal dementia; ALS, Amyotrophic lateral sclerosis; CAP-Gly, cytoskeleton-associated protein-glycine conserved domain; USP, ubiquitin-specific protease domain; Variants firstly were identified in our study (red), and variants were previously reported (black). The corresponding diagnoses are in parentheses.

61.2 ± 10.9 years (range 46–79 years) (Table 1). The frequency of *CYLD* variants in AD was similar to that in FTD, which was 0.99% (10/1008) and 0.95% (1/105), respectively.

Patient No.1 was male and had a family history. At 79 years old, he presented memory decline and loss of orientation. He often forgot what others just said and where things were placed. His mother developed dementia at 80 years and died in her 90s. Brain magnetic resonance imaging (MRI) showed bilateral temporal lobe atrophy. ¹¹C-PiB-positron emission tomography (PET) showed that amyloid β (Aβ) accumulated in the cerebral cortex. He carried the *CYLD* p.Phe288Leu variant. Patient No.2 was a 52-year-old woman with an onset of cognitive impairment at 49 years old, carrying the *CYLD* p.Arg397Ser variant. She was mainly presented with progressive episodic memory loss. Brain MRI revealed moderate hippocampal atrophy. Patient No.3 was a female with dementia starting at 46 years old, and also had *CYLD* p.Arg397Ser variant. She displayed symptoms such as forgetting what just happened and miscalculating money. Gradually, she could not tell the direction of home and got lost. She had no family history. MRI showed whole brain atrophy. Similarly, patient No. 4 was a female with an age of onset of 46 years old and carried *CYLD* p.Arg397Ser variant. She had typical episodic memory impairment. MRI showed bilateral hippocampal atrophy. ¹¹C-PiB-PET revealed that there was diffuse amyloid β (Aβ) deposition in the whole cerebral cortex. Meanwhile, reduced Aβ42 and elevated total-tau and phosphorylated tau were observed in the cerebrospinal fluid (CSF) test.

Patient No.5 was a female who started dementia at 68 years and carried *CYLD* p.Arg397Ser variant. She showed progressive memory loss, such as forgetting put salt in dishes when cooking. Also, she had decreased execution ability. Her brother and mother both presented dementia in their 70s and died several years later. Brain MRI showed bilateral hippocampal atrophy. Patient No.6 was an 80-year-old female who developed dementia at 74 years old and carried *CYLD* p.Tyr485Phe variant. She displayed symptoms such as forgetting what just happened. MRI indicated whole brain atrophy. Patient No.7 was a male with an onset age of 58 years old. He often forgot what others just said and could not recognize his relatives. MRI showed medial temporal atrophy. He finally carried *CYLD* p.Tyr485Phe variant. Patient No. 8 presented at our hospital because of memory loss and loss of orientation. His symptoms began at age of 68 years. Temporal lobes atrophy was observed in brain MRI. PiB-PET showed diffuse Aβ deposition. The genetic screening revealed that he carried *CYLD* p.Tyr485Phe variant. Patient No. 9 was a male with an onset age of 59 years. His father had similar symptoms and died in his 60s. He presented episodic memory impairment, and MRI indicated bilateral temporal and parietal atrophy. He also carried *CYLD* p.Tyr485Phe variant. Patient No. 10 was a 71-year-old patient with an onset age of 70 years, carrying a *CYLD* p.Thr951Ala variant. His sister also had similar symptoms. He presented with marked memory loss, disorientation, and deterioration of visuospatial skills. Brain MRI revealed global brain atrophy and mild leukodystrophy. PiB-PET revealed showed that Aβ was deposited in

Table 1. *CYLD* rare damaging variants identified in AD and FTD patients.

Patient	Gender	Age at onset (years)	Age (years)	Disease duration (years)	Initial symptoms	Family history	MMSE	CDR	MTA
No 1	M	79	81	2	Memory decline	+	8	2	2
No 2	F	49	52	3	Memory decline	–	12	1	3
No 3	F	46	51	5	Memory decline	–	17	1	2
No 4	F	46	48	2	Memory decline	–	9	2	2
No 5	F	68	70	2	Memory decline	+	10	2	3
No 6	F	74	80	6	Memory decline	–	4	3	3
No 7	M	58	60	2	Memory decline	–	7	2	2
No 8	M	68	72	4	Memory decline	–	7	2	3
No 9	M	59	67	8	Memory decline	+	6	3	2
No 10	M	70	71	1	Memory decline	+	16	1	3
No 11	M	56	59	3	Abnormal behavior	–	13	1	1

AD, Alzheimer's disease; FTD, frontotemporal dementia; M, male; F, female; MMSE, Mini-mental State Examination; MoCA, Montreal Cognitive Assessment; CDR, Clinical Dementia Rating; MTA, medial temporal lobe atrophy; PET, positron emission tomography; A β , amyloid β ; MAF, minor allele frequency; P-tau, phosphorylated tau; T-tau, total tau; NA, not available; D, damaging; T, tolerable; B, benign.

the temporal lobe and parietal lobe. Patient No.11 was a male with dementia starting at age of 56 years with a negative family history. He visited our hospital because of abnormal behaviors, such as picking up the garbage and stealing money. Also, he became irritable and often lost his temper for no reason, while his memory remains intact during the disease progression. MRI showed moderate bilateral frontal lobe atrophy, and FDG-PET indicated decreased metabolism in the bilateral frontal lobe while PiB-PET revealed no A β deposits in the cortex. This patient had no signs of amyotrophic lateral sclerosis (ALS). He was finally diagnosed with behavioral variant FTD (bvFTD) and carried the *CYLD* p.Arg397Ser variant.

Discussion

In our study, we identified three novel *CYLD* rare damaging variants (p.Phe288Leu, p.Tyr485Phe, and p.Thr951Ala) and one variant previously reported (p.Arg397Ser) in ten AD patients and one FTD patient. This is the first study of screening *CYLD* variants in AD patients. Also, we are the first to sequence the *CYLD* gene in FTD cases in the Chinese population.

CYLD gene is located in the chromosome 16q12.1 region, encoding a protein including three cytoskeleton-associated protein glycine-rich (CAP-Gly) domains and a ubiquitin-specific protease (USP) domain.¹³ In our study, *CYLD* rare variants were predicted to be damaging by MutationTaster but not by SIFT and PolyPhen2. Although the predictions of these variants were not

consistent, they may still exert effects on AD or FTD development. Firstly, MutationTaster had the best prediction performance when compared to PolyPhen2, Provean, SIFT, and MutationAssessor.²¹ Additionally, in silico tools only predict variants' pathogenicity, and functional studies are warranted to determine their roles in AD or FTD pathogenesis. *CYLD* is a deubiquitinating enzyme targeting ubiquitin chains and regulating the NF- κ B pathway or autophagy.²² In our cohort, two rare variants, including p.Phe288Leu and p.Tyr485Phe, were located within the CAP-Gly domains (residues 232–303 and residues 472–540). Additionally, one rare variant (p.Arg397Ser) is located near the CAP-Gly domain while p.Thr951Ala is near the USP domain (residues 589–947). Cap-Gly domains play important roles in the interaction of *CYLD* and microtubule-associated proteins, such as proteins encoded by *MAPT*.²³ Disruption of the USP domain was associated with loss of *CYLD* catalytic activity.²⁴ Therefore, *CYLD* p.Thr951Ala may regulate *CYLD* catalytic activity. Also, it was speculated that the USP domain accounts for autophagy.¹³ Thus, *CYLD* rare damaging variants we identified may be involved in AD pathogenesis via modulating *CYLD* catalytic activity or its interaction with other microtubule-associated proteins. Our study firstly revealed that *CYLD* rare damaging variants were involved in AD pathogenesis. Interestingly, it is reported that *CYLD* can interact with protein phosphatase 2A (PP2A) and increase PP2A's ability to bind and dephosphorylate Aurora-B.²⁵ PP2A is able to regulate tau phosphorylation,²⁶ therefore, although there are no

PET	APOE	Diagnosis	Variant	Allele and total read depth	GnomAD East Asian non-neuro MAF	Damaging Prediction (MutationTaster/SIFT/PolyPhen2)
Aβ deposition	3/4	AD	c.864 T > A:p.Phe288Leu	40/58	0	D/T/B
NA	3/3	AD	c.1189C > A:p.Arg397Ser	24/63	3.43×10^{-4} (5/14558)	D/T/B
NA	3/3	AD	c.1189C > A:p.Arg397Ser	38/69	3.43×10^{-4} (5/14558)	D/T/B
Aβ deposition	3/3	AD	c.1189C > A:p.Arg397Ser	38/72	3.43×10^{-4} (5/14558)	D/T/B
NA	3/4	AD	c.1189C > A:p.Arg397Ser	29/60	3.43×10^{-4} (5/14558)	D/T/B
NA	3/3	AD	c.1454A > T:p.Tyr485Phe	19/33	0	D/T/B
NA	3/3	AD	c.1454A > T:p.Tyr485Phe	19/37	0	D/T/B
Aβ deposition	3/3	AD	c.1454A > T:p.Tyr485Phe	11/21	0	D/T/B
NA	4/4	AD	c.1454A > T:p.Tyr485Phe	45/91	0	D/T/B
Aβ deposition	3/3	AD	c.2851A > G:p.Thr951Ala	23/45	0	D/D/B
Decreased metabolism in the bilateral frontal lobe No Aβ deposition	3/4	FTD	c.1189C > A:p.Arg397Ser	17/33	3.43×10^{-4} (5/14558)	D/T/B

studies that CYLD promotes PP2A's capacity to regulate the phosphorylation level of tau, it is plausible to suppose that CYLD may be associated with tau's phosphorylation. Furthermore, neuroinflammation is a key pathologic event in AD. Elevated levels of tumor necrosis factor- α (TNF- α) induced necroptosis in HT-22 hippocampal neuronal cells, which was mediated by a signaling pathway that CYLD is involved.²⁷

To date, no studies have shown that *CYLD* is implicated in AD pathogenesis. Our study is the first to reveal that the frequency of *CYLD* rare variants was common in a Chinese AD cohort, in whom approximating 1% of AD patients carried these variants. Our finding indicated that the *CYLD* rare variants probably play a role in AD pathogenesis, and further functional studies are needed to investigate whether CYLD interacts with the proteins encoded by AD pathogenic genes. Furthermore, Dobson-Stone et al. identified that 0.54% (6/1105) of bvFTD patients carried *CYLD* rare variants in Australia, North America, and Europe, while Tábuas-Pereira et al. showed that *CYLD* rare variants accounted for 3.08% (2/65) of FTD patients in a Portuguese cohort.^{11,13} Compared to the two aforementioned studies in the Caucasian population, in our cohort, the frequency of *CYLD* rare variants in FTD patients (1/105, 0.95%) was intermediate, suggesting that population differences may exist.

Some limitations exist in our study. First, some AD patients were diagnosed with probable AD, pathogenic evidence, such as CSF biomarkers or PiB-PET scan, is needed to ensure the diagnosis. Second, the pathogenicity

of rare variants we found was based on bioinformatic analysis, and the functional studies are warranted to confirm their exact roles. Thirdly, only the variants identified in AD or FTD patients were sequenced in controls. Further systematic studies of screening *CYLD* rare variants between patients and controls are needed.

Taken together, we identified four rare damaging *CYLD* variants in 10 AD patients and one FTD patient. We are the first to reveal that *CYLD* rare variants may be implicated in the pathogenesis of AD, which extended the genotype and phenotype of *CYLD*. Additionally, our study further supported the potential role of *CYLD* in FTD pathogenesis.

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Conflict of Interest

Nothing to report.

Author Contributions

Study concept: XX, BJ, and LS. Design: XX, TX, HL, and XL. Acquisition of the data: XL, YZ, LZ, XW, YZ, QY, XH, YL, HJ, and JG. Analysis, or interpretation of data: XX, JW, BT, and JL. Statistical analysis: XX and JL. Drafting of the manuscript: XX. Critical revision of the manuscript for important intellectual content: all authors.

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