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Horizon in frontotemporal lobar degeneration related disorder

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Horizon in frontotemporal lobar degeneration related disorder

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Editorial: Horizon in frontotemporal lobar degeneration related disorder

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KEYWORDS

frontotemporal lobar degeneration (FTLD), amyotrophic lateral sclerosis (ALS), heterogeneity, neuroimaging, molecular imaging, genetics

Editorial on the Research Topic
Horizon in frontotemporal lobar degeneration related disorder

Frontotemporal lobar degeneration (FTLD) is a group of clinically, genetically, and neuropathologically heterogeneous neurodegenerative syndromes characterized by progressive atrophy of the frontal and temporal lobes (1). It can lead to progressive cognitive impairment, with the main clinical characteristics including behavioral abnormalities, personality changes, and impaired social cognitive function, as well as language, executive, and motor dysfunction. In addition to clinical presentation, diagnostic methods emphasize the strong support provided by genetic, neuropsychological, and neuro/molecular imaging tools.

In this Research Topic, we aim to collect and present a series of articles investigating sensitive diagnostic markers associated with the disease and identify its potential pathogenesis. The five articles closely related to the topic emphasized the clinical evidence supporting the diagnosis of FTLD, including data from neuroimaging studies and recent advances in genetics.

Accurate and early diagnosis of patients with FTLD is challenging due to clinical and neuropsychological heterogeneity, as well as atypical and overlapping clinical manifestations. Non-invasive quantitative molecular imaging can assist in better selective visualization of molecular targets *in vivo* for characterizing pathological protein deposition, functional changes, and neuroinflammation in FTLD to support diagnosis. Wang R. et al. discussed advances in molecular imaging in familial FTLD and focused on its implication and prospects in differentiating specific mutations in GRN, MAPT, and C9orf72. Tau-PET using 18F-flortaucipir and 11C-PBB3 demonstrated the elevated tau position in patients with FTLD, which can help differentiate MAPT from GRN or C9orf72 in familial FTLD. In addition, dopamine transporter imaging using 11C-DOPA and 11C-CFT in PET and 123I-FP-CIT in SPECT revealed the disturbance of dopaminergic neurons in both the asymptomatic and symptomatic stages of familial FTLD. PET imaging using 11C-MP4A demonstrated reduced acetylcholinesterase (AChE) activity in patients with FTLD, while PET with 11C-DAA1106 and 11C-PK11195 revealed an increased level of microglial activation associated with neuroinflammation.

Mapping the brain connectome will help to make an early diagnosis and to predict the severity of behavioral variant FTD (bvFTD). Nigro et al. reviewed the related literature on graph theory based on neuroimaging data. The characteristics of brain network organization in bvFTD, semantic variant PPA (svPPA), and non-fluent/agrammatic variant PPA (nfvPPA) were summarized, and the specific global and local brain network changes in patients with FTLD were analyzed. Patients with bvFTD showed a lower mean clustering coefficient, global efficiency, and a higher characteristic path length (2). At the same time, the loss of hubs in

different brain regions of patients with bvFTD, including the frontal gyrus (right superior frontal, inferior orbitofrontal gyri, left anterior cingulate cortex, and cuneus), basal ganglia, and limbic system, has been reported, with new hubs appearing in the orbital frontal and parietal-temporal brain regions. The graphic analysis indicators reflect the neuropsychological characteristics of patients with bvFTD and are associated with clinical symptoms such as cognitive, behavioral, social cognitive and executive impairment, attention disorder, apathy, and inhibition. Due to the limited amount of research, no definite conclusions related to svPPA and nfvPPA have been drawn in this study.

With the concept of ALS-FTD spectrum disorder presented at the International Research Symposium on FTD and ALS held in London, Canada in June 2015 (3), considerable progress has been made in understanding the intersection and overlap between FTD and ALS. In addition to the C9orf72 mutation that has been identified as a key shared pathogenic gene for ALS-FTD, TARDBP, CHCHD10, FUS, TBK1, SQSTM1, UBQLN2, and VCP, mutations have also been associated with ALS-FTD (4). Clinically, ALS and FTD are frequently found in the same family. The study by Tábuas-Pereira et al. included 57 carriers of the pathogenic mutation without GRN, MAPT, or C9orf72 pathogenic variants. Rare mutations, including ERBB4, ANG, CHRNA4, CHRNB4, SETX, and GLT8D1, were found and predicted to be pathogenic in genes previously associated only with ALS, providing additional evidence that the ALS gene may also be involved in the pathogenesis of FTD.

Based on a cohort of Chinese ALS patients, Feng et al. completed pathogenic gene screening using second-generation sequencing technology, reported the first VCP mutation carrier of PDB showing ALS in Chinese population, and detailed the clinical characteristics of 3 ALS patients carrying the VCP p.R155C mutation. In this review, ALS patients with VCP p.R155C mutations tended to develop at a relatively young age, presenting with symmetrical proximal muscle weakness in the arms or legs, and then progressing to the distal limb muscles. ANXA11 pathogenic genes were investigated in the Chinese ALS and/or FTD cohort by Wang Y. et al. and the authors found a heterozygous mutation of ANXA11 (c.119A>G, p.D40G). This type of mutation was previously thought to be only associated with ALS and was first identified in ALS-FTD (5). A literature review found that patients with the same D40G mutation had different clinical symptoms. The clinical heterogeneity of ANXA11 mutation-related diseases is high, and further research is needed to advance this area.

In summary, FTLD is a heterogeneous disorder with a wide range of clinical, genetic, and neuropathological features. Our Research Topic presents the latest evidence from recent genetic and neuroimaging findings, focusing on the validation of proposed biomarkers from new perspectives and the discovery of new candidates. In the absence of clear, sensitive, and early biomarkers, it is suggested that a framework of diagnosis be established based on effective clinical criteria and practical and readily available diagnostic methods.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Plasma Uric Acid Helps Predict Cognitive Impairment in Patients With Amyotrophic Lateral Sclerosis

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Objective: Uric acid as an antioxidant plays an important role in neurodegenerative disease. Our objective is to investigate the relationship between plasma uric acid and cognitive impairment in patients with amyotrophic lateral sclerosis (ALS).

Methods: In this cross-sectional study, 124 ALS patients were screened by the Edinburgh Cognitive and Behavioral Screen (ECAS) and classified according to the revised Strong's criteria. Additionally, based on total ECAS cut-off score patients were categorized into those with cognitive impairment (ALS-cie) and those without cognitive impairment (ALS-ncie), and clinical data and uric acid level were compared between the two groups. Parameters with significant differences were further included in a multivariate linear regression analysis with ECAS score as a dependent variable. Hold-out validation was performed to evaluate the fitness of regression model.

Results: Up to 60% of ALS patients showed cognitive or/and behavioral impairment. The ALS-cie group had lower education level ($p < 0.001$), older age at symptom onset ($p = 0.001$), older age at testing ($p = 0.001$), and lower plasma uric acid ($p = 0.01$). Multivariate analysis showed increased uric acid ($\beta = 0.214$, $p = 0.01$), lower age at testing ($\beta = -0.378$, $p < 0.001$), and higher education level ($\beta = 0.424$, $p < 0.001$) could predict higher ECAS score ($F = 19.104$, $R^2 = 0.381$, $p < 0.0001$). Validation analysis showed that predicted ECAS score was significantly correlated with raw ECAS score in both the training set ($rs = 0.621$, $p < 0.001$) and the testing set ($rs = 0.666$, $p < 0.001$).

Conclusions: Cognitive impairment was a common feature in our Chinese ALS patients. Plasma uric acid might help evaluate the risk of cognitive impairment in ALS patients when combined with education level and age at testing.

Keywords: amyotrophic lateral sclerosis, cognitive impairment, biomarker, uric acid, ECAS

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disorder characterized by progressive loss of upper and lower motor neurons. Although ALS was initially considered only to involve the motor system, cognitive impairment has been increasingly recognized as one feature of ALS (1). While up to half of ALS patients had mild cognitive impairment, 15% of patients fulfilled

the diagnostic criteria of frontotemporal dementia (FTD) (2, 3). The overlapping clinical (4), neuroimaging (5), neuropathological (6), and genetic features (7) suggested that ALS and FTD might constitute a disease spectrum. Since cognitive impairment could increase the caregiver burden and shorten survival (8), early detection of cognitive abnormalities in ALS patients is essential.

Diagnosis of cognitive impairment has been largely based on comprehensive neuropsychological tests (9), which could be limited by resource and time. Additionally, completion of those tests might be restricted by the physical disabilities of ALS patients. In contrast, blood biomarkers have the potential of being readily available. One study on hormonal peptides showed that plasma neuropeptide Y and leptin levels might be markers of cognitive changes measured by Addenbrooke's Cognitive Examination-Revised in ALS patients (10). Another study found significant differences in the plasma levels of 20 proteins by mass spectrometry analysis in 36 ALS patients with or without cognitive impairment using Addenbrooke's Cognitive Examination-III (11).

Oxidative stress was involved in pathological processes leading to neuronal damage in ALS (12). The neuroprotective role of plasma uric acid as an antioxidant has been studied widely in neurodegenerative diseases including ALS (13). Two case-control studies showed that ALS patients had significantly lower plasma uric acid than healthy controls, and the lower uric acid level was associated with faster disease progression (14, 15). Another two randomized clinical trials reported that ALS patients with a higher uric acid level at baseline had prolonged survival advantages (16, 17). Additionally, the uric acid level was reversely correlated with disease stages and significantly decreased during disease progression (18). Furthermore, a prospective study involving 319,617 participants found that uric acid level was inversely related to ALS risk in healthy individuals (19). However, the relationship between plasma uric acid level and cognitive impairment of ALS patients is still unknown.

In this study, we aimed to identify whether plasma uric acid could help evaluate cognitive impairment in Chinese ALS patients.

METHODS

Participants

One hundred and ninety ALS patients (possible, probable, or definite ALS according to the El Escorial criteria) (20) who were admitted to Department of Neurology, Tongji Hospital in Wuhan between August 2017 and October 2020 were screened for this cross-sectional study. Exclusion criteria included other neurological disorders affecting cognitive function, such as stroke, traumatic brain injury, epilepsy; psychiatric disorders; major organ dysfunction; loss of both language and writing ability, and alcohol and drug abuse. Out of the 190 patients, 124 patients (65.3%) were finally included in this study (**Figure 1**). The study was approved by the Ethics Committee of Tongji Hospital, and all patients provided written informed consent to participate.

Data Acquisition

Clinical information including sex, age, disease duration, education time, body mass index (BMI), and site of onset was collected. Patients were evaluated at their first visit by the Chinese version of Edinburgh Cognitive and Behavioral Screen (ECAS) (21), a screening tool for the comprehensive assessment of cognitive status of ALS patients. It consists of five cognitive domains, including language (28 points), executive functions (48 points), verbal fluency (24 points), memory (24 points), and visuospatial functions (12 points), which make up a total score of 136 points (21, 22). The cut-off total score (81.92 points) and subdomains scores were calculated as two standard deviations below the corresponding mean score of the healthy Chinese population (22). Additionally, ECAS includes a behavioral assessment of patients by caregivers (10 points), based on the key behavioral criteria of diagnosing behavioral variant FTD (21). It evaluates five behavioral domains including disinhibition (three points), apathy (one point), loss of sympathy (two points), perseveration (two points), and changes in eating behaviors (two points), as well as psychotic symptoms (three points). The severity of physical disability was measured by the amyotrophic lateral sclerosis functional rating scale-revised (ALSFRS-R), which evaluated bulbar, upper limb, lower limb, and respiratory function, with a higher score (total score 48 points) representing better physical function (23). Plasma uric acid level was measured via enzymatic colorimetric method using the Cobas c701 automatic analyzer (Roche) in the Department of Clinical Laboratory of Tongji Hospital.

Classification

All patients were screened into two groups based on total ECAS score, i.e., the group with cognitive impairment (ALS-cie group, total score <81.92) and the group without cognitive impairment (ALS-ncie group, total score ≥ 81.92) (22). Additionally, patients with abnormality in at least one behavioral domain were considered to have behavioral abnormality.

Patients were further categorized into five classical groups according to the revised Strong's criteria: (1) ALS with cognitive impairment (ALSci), if patients had at least one abnormal symptom in the domain of language, executive function, or verbal fluency; (2) ALS with behavioral impairment (ALSbi), if patients had apathy or at least two other behavioral/psychotic symptoms; (3) ALS with combined cognitive and behavioral impairment (ALScbi), if patients met the criteria of both ALSci and ALSbi; (4) ALS-FTD, if patients had at least three abnormal symptoms of cognitive or behavior domain which were progressive; (5) ALS with normal cognition (ALSns), if patients did not meet any of the above mentioned criteria (24).

Statistical Analysis

All statistical analyses were performed by SPSS statistical software (version 22.0). Normally distributed data were presented as the mean $\pm$ SD, while non-normally distributed data were showed as median (range). The distribution of categorical data was

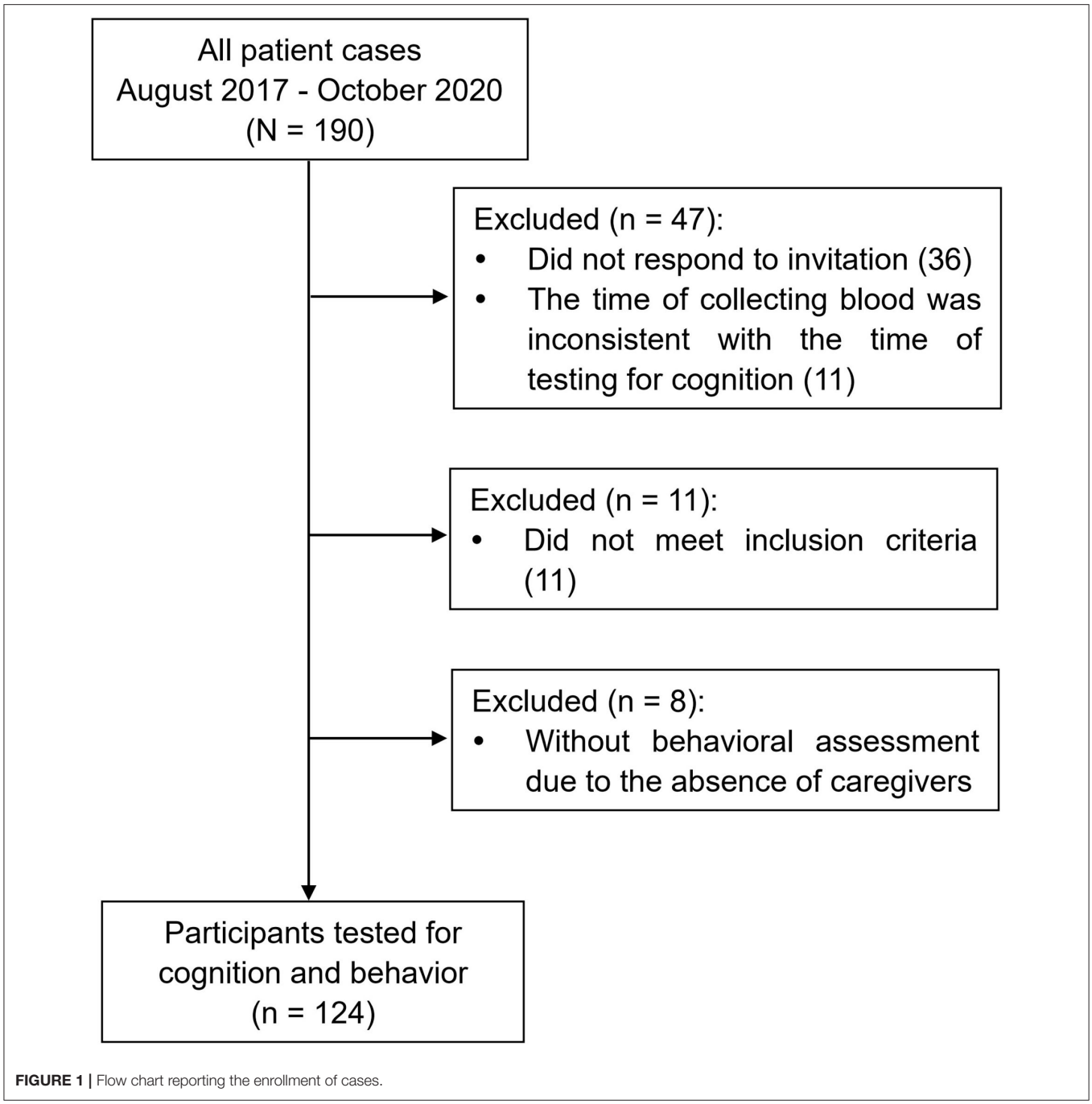

FIGURE 1 | Flow chart reporting the enrollment of cases.

reported as frequencies and percentages. To compare clinical parameters and uric acid level between the two groups, the Chi-square test was used for categorical data, the independent *t*-test was used for normally distributed continuous data, and the Mann-Whitney-U-test for non-normally distributed continuous data. Significantly different parameters were further included in a multivariate linear regression analysis as independent variables, with the total ECAS score as a dependent variable. Education level was dichotomized into lower education ($\leq$9 years) and higher education (>9 years) according to the median value. Moreover, the hold-out validation analysis was performed to assess the fitness of regression model by dividing patients randomly into two subsets, i.e., 80% as training set and 20% as testing set. After obtaining predicted ECAS scores through the regression model, correlations between predicted and raw ECAS scores were calculated in both training set and testing set. Additionally, correlations between plasma uric acid level and ECAS total/subdomain scores were analyzed by Pearson correlation or Spearman correlation in case of normally distributed data or non-normally distributed data, respectively.

TABLE 1 | Clinical characteristics of ALS patients.

	All patients n =124	ALS-ncie n = 76	ALS-cie n = 48	P-value
Male (%)	58	63	50	0.15[a]
Education (year)	9 (0–16)	11 (4–16)	7.5 (0–12)	<0.001[c]
Age at onset (year)	53.6 ± 10.8	51.1 ± 10.4	57.5 ± 10.4	0.001[b]
Age at testing (year)	54.6 ± 10.9	52.1 ± 10.5	58.6 ± 10.3	0.001[b]
Duration of illness (month)	11 (1–64)	10.5 (2–49)	11 (1–64)	0.41[c]
BMI (kg/m^2)	21.6 (15.2–28.3)	21.6 (15.2–28.3)	21.6 (17.3–26.9)	0.93[c]
Site of onset: bulbar/limb/respiratory/mixed (%)	18/75/1/6	16/78/1/5	21/71/0/8	0.57[a]
ALSFRS-R score	41 (15–48)	41.5 (19–48)	40 (15–47)	0.57[c]
Uric acid (μmol/L)	310.4 ± 76.0	323.8 ± 77.1	289.3 ± 70.0	0.01[b]

ALS-cie, amyotrophic lateral sclerosis with cognitive impairment; ALS-ncie, amyotrophic lateral sclerosis without cognitive impairment; BMI, body mass index; ALSFRS-R, amyotrophic lateral sclerosis functional rating scale-revised.

[a] *χ^2-test.*

[b] *Independent t-test with values presented as mean ± SD.*

[c] *Mann-Whitney-U-test with values presented as median (range).*

All statistical analyses were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Clinical Characteristics

Of 124 ALS patients, 52 (41.9%) were women, and 72 (58.1%) were men. The mean age at onset was 53.6 ± 10.8 years, and the mean age at testing was 54.6 ± 10.9 years. The site of onset was limb onset in 93 patients (75%), followed by bulbar onset in 22 patients (17.7%), mixed onset in 8 patients (6.5%), and respiratory onset in 1 patient (0.8%). The median education time was 9 years, the median duration of illness was 11 months, the median BMI was 21.6, and the median ALSFRS-R score was 41 (**Table 1**).

Patients in the ALS-cie group had shorter education time (7.5 vs. 11 years, $p < 0.001$), older age at symptom onset (57.5 ± 10.4 vs. 51.1 ± 10.4 years, $p = 0.001$), older age at testing (58.6 ± 10.3 vs. 52.1 ± 10.5 years, $p = 0.001$), and lower plasma uric acid level (289.3 ± 70.0 vs. 323.8 ± 77.1, $p = 0.01$) than those in the ALS-ncie group. Sex ratio, disease duration, site of onset, BMI values, and ALSFRS-R score did not differ between the two groups (**Table 1**).

Cognitive and Behavioral Status of ALS Patients

Forty-eight (38.7%) patients had abnormal ECAS total scores based on a cut-off value of 81.92. Of five cognitive domains, executive impairment was the most frequent (46.8%), followed by visuospatial (39.5%) and memory (29.0%) impairment. Around one-fifth of patients had language (25.8%) and verbal fluency (21.0%) impairment (**Figure 2A**).

Behavioral and psychotic abnormalities were observed in 39 (31.5%) of patients, with 21 (16.9%) showing abnormalities in one, 10 (8.1%) in two, and 7 (5.6%) in three or more domains. Apathy (16.9%) and loss of sympathy (16.9%) were the most common, followed by psychotic symptoms (7.3%), disinhibition (5.6%), changes in eating behaviors (4.8%), and perseveration (4.0%) (**Figure 2B**).

According to the revised Strong's criteria, 49 patients (39.5%) had ALSns, 49 (39.5%) patients had ALSci, 13 (10.5%) had ALS-FTD, 8 (6.5%) had ALSbi, and 5 (4.0%) had ALScbi (**Figure 2C**).

The Relationship Between Plasma Uric Acid and Cognitive Subdomains

Plasma uric acid level was weakly associated with scores of language ($rs = 0.268$, $p = 0.003$) and executive domain ($rs = 0.179$, $p = 0.047$). No significant correlations between uric acid and verbal fluency, memory, and visuospatial functions were identified (**Table 2**).

Multivariate Regression Analysis and Model Validation

Education level, age at testing (age at onset was not selected due to their collinearity relationship), and plasma uric acid level were included in a lineal regression analysis. Increased uric acid ($\beta = 0.214$, $p = 0.01$), lower age at testing ($\beta = -0.378$, $p < 0.001$), and higher education level ($\beta = 0.424$, $p < 0.001$) were significant predictors of higher ECAS score ($F = 19.104$, $R^2 = 0.381$, $p < 0.0001$) (**Table 3**). Validation analysis showed predicted ECAS scores were significantly correlated with raw ECAS scores in both the training set ($rs = 0.621$, $p < 0.001$) and the testing set ($rs = 0.666$, $p < 0.001$), indicating reasonable model fitness.

DISCUSSION

Our findings for the first time showed that cognitive or behavioral dysfunction occurred in around 60% of Chinese ALS patients according to the revised Strong's criteria (24). Among all cognitive and behavioral subdomains, executive dysfunction was the most common subtype of cognitive impairment, while apathy and loss of sympathy were the main subtypes of behavioral abnormality. Importantly, we found that low plasma uric acid

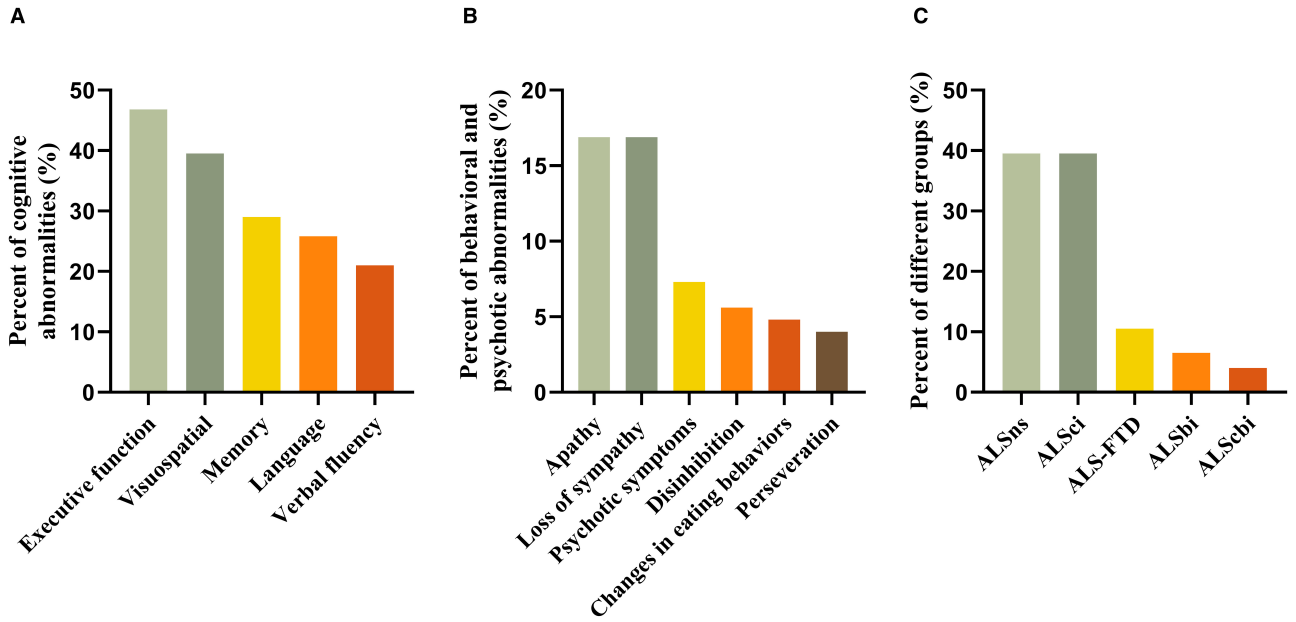

FIGURE 2 | (A–C) Frequency of cognitive and behavioral subdomain impairment as well as ALS grouping according to the revised Strong's criteria.

TABLE 2 | The relationship between plasma uric acid and cognitive domains.

Cognitive subdomains	*rs*	*P*-value
Language	0.268	0.003
Fluency	0.068	0.456
Executive functions	0.179	0.047
Memory	0.147	0.103
Visuospatial functions	0.14	0.121

rs, Spearman's relational coefficient.

TABLE 3 | Multivariate model of the total ECAS score.

Variable	*B*	*β*	95% CI	*P*-value
Education level	21.324	0.424	13.145 to 29.503	<0.001
Age at testing, years	−0.854	−0.378	−1.207 to −0.483	<0.001
Uric acid, μmol/L	0.07	0.214	0.017 to 0.123	0.010

B, regression coefficient; β, standardized regression coefficient; CI, confidence interval.

could help predict cognitive impairment in ALS patients along with aging and low educational level.

While the percentages of ALSbi and ALScbi in our ALS cohort were similar to previously reported data, ALSci (39.5% vs. around 16%) was more than doubled and ALS-FTD (10.5% vs. around 20%) was around half compared to the Italian populations (mean age 66) (25, 26). Another study showed the percentage of overall cognitive impairment (36 vs. 22%, ECAS total cut-off score) as well as executive dysfunction (47 vs. 20%) is higher among the ALS patients from China (mean age 55) than those from Germany (mean age 60), while the authors stated that the language used in ECAS questionnaire could not fully account for the differences (27). Interestingly, the percentage of cognitive dysfunction from our ALS cohort (mean age 55) based on the ECAS criteria was highly consistent with the data of Chinese patients recruited in another major academic center (22). Although other factors including educational level might influence ECAS performance, these findings support that race related differences in neurobiological networks might play a role in the different performances of Chinese and Caucasian populations (27). While executive dysfunction was the most common cognitive change in ALS patients consistent with previous studies (22), our study found that cognitive domains including memory and visual space were also frequently affected (28, 29). While some studies proposed that memory impairment was due to the failure of encoding secondary to executive impairment (30), a recent finding suggested that memory impairment was a primary dysfunction due to temporal lobe involvement in ALS patients (31). Similarly, poor visuospatial performance might be associated with temporal lobe involvement (32).

Our ALS patients were significantly younger and had lower bulbar onset percentage (18 vs. 33%) than the Italian cohort (53.6 vs. 65.5 years), which could contribute to the lower prevalence of ALS-FTD (26). In addition, C9orf72 gene mutation was strongly associated with ALS-FTD (26). The lower percentage of ALS-FTD in our ALS cohort could be partly attributed to the significantly lower prevalence of C9orf72 gene mutation in the Chinese than the Caucasian populations (33). In line with previous studies (34–36), apathy and loss of sympathy were common behavioral changes in ALS patients.

Our study showed lower plasma uric acid level was an independent predictor of cognitive impairment. Additionally, plasma uric acid level was positively, although weakly, correlated with executive and language domains of ECAS. Uric acid is the end-product of purine metabolism (37). Hyperuricemia is associate with gout with the deposition of monosodium urate in joints (38). Additionally, uric acid has been shown to act as a major antioxidant in the human body by scavenging reactive oxygen species (ROS) and peroxynitrite (39) and inhibiting iron-mediated oxidation through chelation (40). A prospective study involving 4,618 participants aged 55 years or older found that higher plasma uric acid level was associated with better cognitive function and a decreased risk of dementia after adjusting for cardiovascular risk factors (41). Another study conducted on 111 patients with tauopathies, including FTD, Alzheimer's disease (AD), and progressive supranuclear palsy, found lower plasma uric acid in patients compared to healthy controls and demonstrated that plasma uric acid level was inversely associated with risk of tauopathies independent from age and gender (42). One previous study using astroglial cultures showed that uric acid could boost glutathione production through astrocytic molecular pathways (43) and might play a protective role in reducing oxidative stress in tauopathies (44).

The most frequently identified pathology in the ALS-frontotemporal spectrum disorder is the accumulation of transactive response DNA-binding protein 43 (TDP-43) in the cytoplasm of neurons (6). One study found a significant difference in the severity of TDP-43 pathology between ALS-FTD and non-demented ALS patients (45). Another study further confirmed that ALS-specific cognitive impairment of verbal fluency, language, and executive function based on ECAS was highly correlated with TDP-43 pathology in the corresponding functional lobal areas in ALS patients without clinically diagnosed dementia (46). A post-mortem ALS study found that TDP-43 aggregation showed temporal progression patterns across different disease stages with the TDP-43 pathology expanding from the motor cortex to the prefrontal and temporal lobe (47).

Of note, mitochondria dysfunction and oxidative stress have been reported to play an important role in TDP-43 pathology of cellular and animal models (48, 49). Basic *in vivo* and *in vitro* studies showed that TDP-43 could induce mitochondrial ROS production and negatively affect neuronal survival and function (50). The nuclear factor erythroid 2-related factor 2 (Nrf2) was a main regulatory factor in preventing the accumulation of ROS and reducing oxidative stress (51). Overexpression of Nrf2 in astrocytes could delay symptom onset and prolong survival in ALS mouse models (52). Protective effects of uric acid on motor neurons could be exerted by activating Nrf2 expression in ALS models, leading to increased glutathione, and decreased oxidative damage (53). Furthermore, deficiency of Nrf2 expression could aggravate the impairment of recognition memory in a neuroinflammatory mouse model (54), while induction of Nrf2 expression alleviated cognitive impairment in an AD mouse model (55). We thus postulate that higher plasma uric acid level could help attenuate ROS production and oxidative stress in brain areas critical to maintaining normal cognition in ALS patients. One study proposed that lower uric acid levels in ALS patients might be due to malnutrition attributed to the bulbar onset and longer disease duration (56). Our study showed no difference in BMI values, disease duration, ALSFRS-R score, and bulbar onset between ALS-ncie and ALS-cie, thus not supporting a correlation between nutrition and uric acid level in our ALS patients. Inosine, the urate precursor, was proven to increase serum uric acid level safely and tolerably when administered orally or via feeding tubes in a small pilot trial of ALS patients (57). It would be interesting to explore whether inosine helps prevent or mitigate cognitive decline in ALS patients in future longitudinal studies.

The current study had several limitations. The cognitive and behavioral status of our patients was only evaluated by ECAS, thus not fully fulfilling the technical requirements of the revised Strong's criteria for accurate classification, for example, primary progressive aphasia and loss of insight could not be evaluated in a standardized way. Our study has a small sample size due to the low incidence of ALS and all participants were recruited in a single tertiary center, making referral bias possible. In addition, our study had a cross-section design and plasma uric acid was not dynamically measured, weakening its predictive strength. Furthermore, genetic features including C9orf72 mutation status that could affect the cognitive and behavioral status of ALS patients (26, 58) were not obtained due to limited resources. Of note, age was one of the major factors affecting cognitive function. It had been showed that hypertension, diabetes, cardiovascular disease, smoking, systemic inflammation, and stress were associated with cognitive impairment during the aging process (59), and these factors were not included and comprehensively evaluated in our regression model. Lastly, we did not have the neuroimaging data to explore potential correlation between cognitive/behavioral status and specific brain areas' structural or functional changes (47).

CONCLUSIONS

Cognitive impairment was a common feature in the Chinese ALS patients. Decreased plasma uric acid was an independent risk factor of cognitive dysfunction in ALS patients apart from aging and low educational level. Longitudinal studies on the dynamic changes in plasma uric acid and cognitive status of ALS patients could help further clarify their relationship during disease progression which might provide potential therapeutic options.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Tongji Hospital. The

patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JT, MZ, and FD contributed to the study design. JT, YY, ZG, ZL, and LH contributed to data acquisition. JT and ML contributed to data interpretation and statistical analysis. JT drafted the manuscript. ML and MZ revised the manuscript. All authors contributed to the article and approved the submitted version.

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Phenotype of VCP Mutations in Chinese Amyotrophic Lateral Sclerosis Patients

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Mutations in the valosin-containing protein (VCP) gene have been linked to amyotrophic lateral sclerosis (ALS) in the Caucasian populations. However, the phenotype of VCP mutations in Chinese patients with (ALS) remains unclear. Targeted next-generation sequencing covered 28 ALS-related genes including the VCP gene was undertaken to screen in a Chinese cohort of 275 sporadic ALS cases and 15 familial ALS pedigrees. An extensive literature review was performed to identify all patients with ALS carrying VCP mutations previously reported. The clinical characteristics and genetic features of ALS patients with VCP mutations were reviewed. One known p.R155C mutation in the VCP gene was detected in two siblings from a familial ALS pedigree and two sporadic individuals. In addition, the same VCP p.R155C mutation was detected in an additional patient with ALS referred in 2021. Three patients with VCP p.R155C mutation presented with muscular weakness starting from proximal extremities to distal extremities. The other patient developed a phenotype of Paget's disease of bone in addition to the progressive muscular atrophy. We reported the first VCP mutation carrier manifesting ALS with Paget's disease of bone in the Chinese population. Our findings expand the phenotypic spectrum of the VCP mutations in Chinese patients with ALS and suggest that ALS patients with VCP p.R155C mutations tend to present with relatively young onset, symmetrical involvement of proximal muscles weakness of arms or legs, and then progressed to distal muscles of limbs.

Keywords: amyotrophic lateral sclerosis, Paget's disease of bone, valosin-containing protein, R155C, phenotype

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by motor dysfunction in limbs (muscle weakness, atrophy, and spasticity) and bulbar palsy, such as dysarthria and dysphagia. Cognitive decline or behavioral impairment occurs in some cases. Approximate two-thirds of patients with ALS had a limb onset. The typical disease course of ALS cases is aggressive, ending in death due to respiratory failure within 3–5 years (1).

Approximately 5–10% of ALS cases present in a familial pattern, while the others have no family history. The genetic background of ALS is complicated, correlated with a growing spectrum of genes, such as C9orf72, SOD1, FUS, and TDP-43 (1).

Valosin-containing protein (VCP) gene codes a highly conserved triple A-adenosine triphosphatase (triple A-ATPase), which operates as a regulatory factor in the procedure of endoplasmic reticulum-associated degradation (2). When the triple A-ATPase has functional deficits, the ubiquitin-dependent recycling or degradation by the proteasome will be disrupted. Additionally, the process of membrane fusion, transcriptional activation, and apoptosis heavily relies on the VCP-coded ATPase (3). Mutations in the VCP gene have been determined as a causative gene of the syndrome, inclusion body myopathy (IBM) with Paget's disease of bone (PDB), and frontotemporal dementia (FTD) (IBMPFD) since 2001 (4). The clinical spectrum of VCP gene-related diseases was expanded to ALS when a four-generation Italian ALS pedigree with VCP mutation was detected by whole-exome sequencing in 2010 (5). Subsequent studies in Caucasian populations detected VCP mutations in both familial and sporadic patients with ALS (5, 6). Mutations in the VCP gene in patients with ALS of Chinese origin have been rarely reported (7). Here, we reported the phenotype of four patients with ALS carrying VCP mutation of Chinese origin.

MATERIALS AND METHODS

Subjects

In total, a cohort of 275 sporadic ALS cases and 15 familial ALS pedigrees was recruited at Fujian Medical University Union Hospital and Henan Provincial People's Hospital between January 2017 and December 2018. Another sporadic ALS case referred to Fujian Medical University Union Hospital in 2021 was included. Diagnosis of ALS was made according to the revised El Escorial criteria (8). Familial ALS was diagnosed if one or more first- or second-degree relatives developed ALS. The study was approved by the Ethics Committee of Fujian Medical University Union Hospital and Henan Provincial People's Hospital. All subjects involved in this research have offered written consent.

Genetic Studies

Genomic DNA extracted from venous peripheral blood lymphocytes of both sporadic cases and the proband of the family were subjected to the targeted next-generation sequencing on Illumina Hiseq sequencer (Illumina Inc., San Diego, CA, USA). An ALS-specific gene panel which included 28 genes (SOD1, FUS, TARDBP, VCP, VAPB, SPG11, OPTN, PFN1, ANG, ALS2, DAO, UBQLN2, SIGMAR1, SETX, FIG4, DCTN1, TUBA4A, TBK1, SQSTM1, CHCHD10, MATR3, HNRNPA1, HNRNPA2B1, KIF5A, ANXA11, TIA1, CCNF, and NEK1) was designed. The targeted regions were designed to include all exons and flanking regions of the 29 genes which contained the VCP gene (NM_007126.5). The GGGGCC expansions in C9orf72 were screened as previously described (9).

As a result of sequencing, the mean on-target coverage was 880× with an average percentage of targets covered greater or equal to 100× of 100%. Variant filtering process has been described (10). The identified variants were subsequently validated by Sanger sequencing. Bioinformatic analysis of the variants was performed as previously described (10).

Literature Review

We conducted a literature search in Medline to identify previous studies that screened for VCP mutations in patients with ALS. The following keywords were used: "Valosin-containing protein" OR "VCP", in combination with "amyotrophic lateral sclerosis" OR "ALS" OR "motor neuron(e) disease" OR "MND". Only English language literature was included in the review. For each eligible publication, the following information was extracted: name of the **first** author, publication year, population, the sample size of familial ALS (FALS), and/or sporadic ALS (SALS), numbers of VCP mutation carriers in FALS and/or SALS. For patients with ALS carrying VCP mutations, the following information was extracted from the relevant papers: **first** author, year of study, population, sex, age, family history, clinical features, and genetic characteristics.

Statistical Analysis

In each study, the mutation frequencies of the VCP gene were reported as the number of the mutation carriers among all cases of FALS or SALS screened. Single case reports, such as the additional VCP p.R155C mutated ALS referred to our center in 2021 were not included in the meta-analysis. The mutation frequencies in different populations were combined using a fixed-effects model. A statistical analysis was carried out using the Meta function of R (R version 3.64) (https://www.r-project.org/).

RESULTS

Clinical Features of the ALS Cohort

Between January 2017 and December 2018, 275 sporadic cases and 15 familial pedigrees meeting the diagnostic criteria of ALS were enrolled in our study. There were 173 men and 117 women with mean onset age of 55.3 years (SD, 11.6). Of the total cases, 19.3% of patients reported a bulbar onset, 79.0% had a limb onset, and 1.7% had a respiratory onset.

Genetic Analysis

One known heterozygous missense mutation in the VCP gene, c.463C>T (p.R155C) (**Figure 1A**) was identified in one familial ALS proband (III-6) and his affected sister (III-5) (**Figure 1B**), as well as another sporadic patient in the cohort of 275 sporadic ALS cases and 15 familial ALS pedigrees. The same VCP p.R155C mutation was detected in the additional patient referred to Fujian Medical University Union Hospital in 2021. No parental DNA samples of patient 3 and patient 4 were available for sequencing. No variants in other ALS-related genes were identified in these patients.

Clinical Features of Patients With VCP p.R155C Mutation

Clinical features of the four patients carrying VCP p.R155C mutations in this study are summarized in **Table 1**.

The familial ALS proband (III-6, Patient 1) was a 51-year-old male with progressive weakness in four limbs. At the age of 42 years, he complained of weakness in the lower limbs. He felt clumsy when climbing upstairs and unusual fatigue when walking a long distance. He began to have difficulties in uplifting

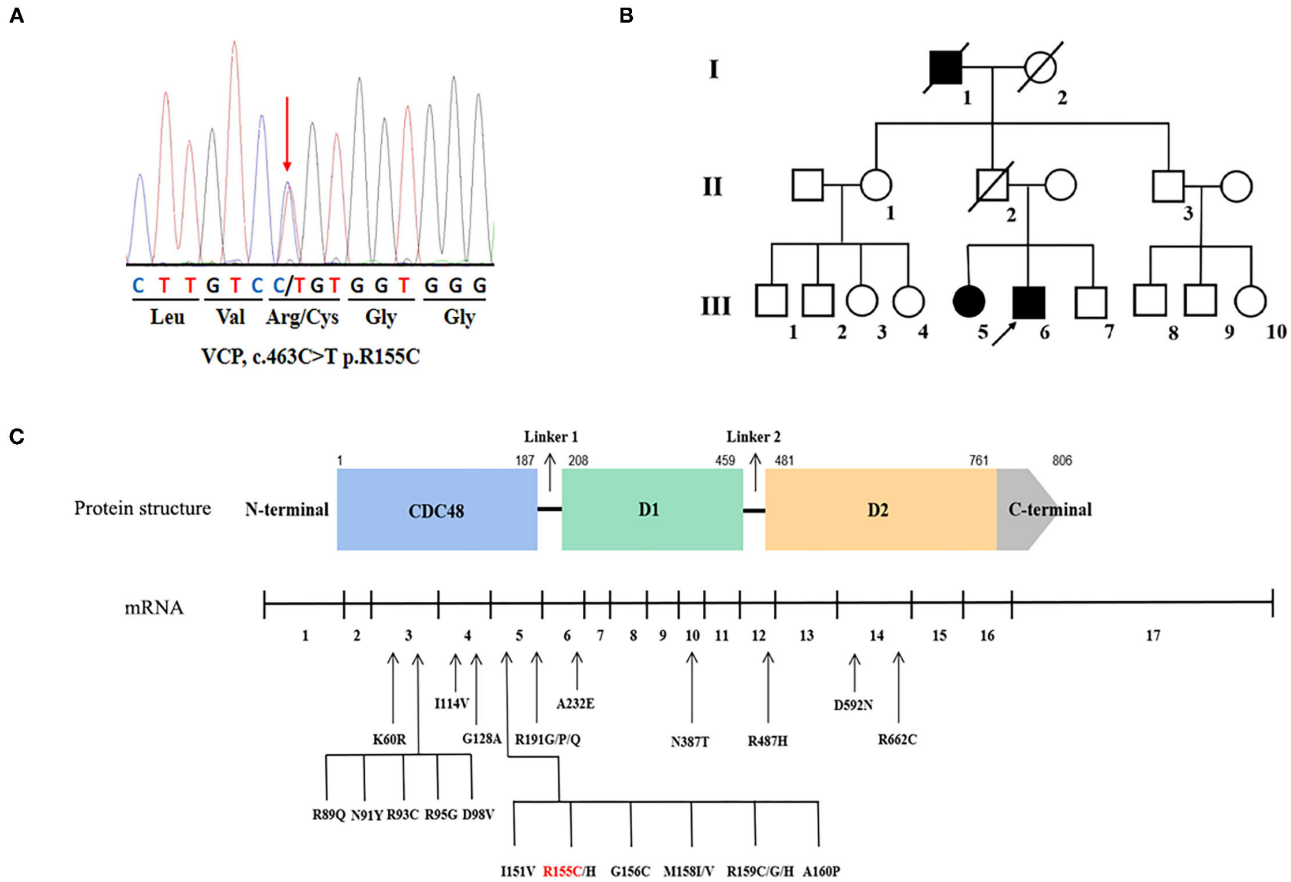

FIGURE 1 | Genetic information of the valosin-containing protein (VCP) mutation in our study and overview of the VCP mutations associated with amyotrophic lateral sclerosis (ALS). **(A)** Sequencing chromatograms of the VCP p.R155C mutation. **(B)** The pedigree of the familial ALS with VCP p.R155C mutation. Arrow indicates the index patient. **(C)** Schematic graph of the VCP protein and overview of the VCP mutations linked to ALS. The locations of mutations are depicted in the mRNA structure where exons are numbered 1–17.

TABLE 1 | Clinical features of the amyotrophic lateral sclerosis (ALS) patients with valosin-containing protein (VCP) p.R155C mutation in this study.

Case	Gender	Age of onset (years)	Site at onset	Bulbar symptom	Phenotype	Additional symptoms	Disease progression (ΔALSFRS-R/m)	Disease duration (month)
Patient 1 III-6	Male	41	Lower limbs	Yes	Classic ALS	No	0.2	>116 (alive)
Patient 2 III-5	Female	51	Lower limbs	No	Classic ALS	No	0.2	>42 (alive)
Patient 3	Male	48	Lower limbs	No	PMA	No	0.2	>102 (alive)
Patient 4	Male	44	Lower limbs	No	PMA	PDB	0.2	>45 (alive)

ALSFRS-R, revised ALS functional rating scale; PDB, Paget's disease of bone; PMA, progressive muscular atrophy.

his arms 4 years later. In the following years, he developed weakness of hands. No cognitive deficits or behavioral changes were noted. The neurologic examination at 7 years after onset revealed obvious weakness and atrophy of four limbs (MRC 4/5), more severe in the proximal muscles of limbs, with diffused reduction of deep tendon reflexes. Babinski's sign was elicited bilaterally. The sensory system and cognition were not affected. Serum creatine kinase (CK) level was normal. Electromyography (EMG) demonstrated fibrillations and positive sharp waves in muscles of four limbs and left thoracic paravertebral muscles, with muscle unit action potentials of increased amplitude, prolonged duration, and reduced recruitment in muscles of limbs. The ALS Functional Rating Scale-Revised (ALSFRS-R) score was 39/48. The score of Montreal Cognitive Assessment (MoCA) testing was 27/30. He developed exertional dyspnea 9 years after onset and began to use the non-invasive positive-pressure ventilation. At the last follow-up at 116 months after onset, he could still walk slowly with help and do daily life activities without assistance. He had neither dysarthria nor dysphagia. A repeated ALSFRS-R score was 30 with an estimated

progression rate of 0.2 score/month since symptom onset. The sister of proband (III-5, Patient 2) had paraparesis in the legs since she was 51 years. She only had some trouble running and climbing upstairs. Neurological examination at 2 years after onset revealed mild atrophy and weakness of lower limbs (MRC 4/5). The deep tendon reflexes were brisk in four limbs. Hoffmann's sign and Babinski's sign were elicited bilaterally, with no sensory and bulbar involvement. Serum CK level was normal. EMG revealed acute and chronic neurogenic changes in all limbs and thoracic muscles. The ALSFRS-R score was 46/48. The score of MoCA testing was 28/30. At the last follow-up at 42 months after onset, she could still walk and go upstairs slowly with a cane, without the involvement of upper limbs. The ALSFRS-R score was 41 with an estimated progression rate of 0.2 score/month since symptom onset. Their father (II-2) died of acute myocardial infarction at the age of 72 years without evident symptoms of limb weakness, and their unaffected mother was still alive. The grandfather (I-1) of the proband exhibited signs of weakness in lower limbs and stayed bedridden for over 10 years before he died in his 70s. However, he was not formally diagnosed with ALS.

Patient 3 was a 57-year-old male who noticed weakness in the legs at the age of 48 years. Initially, he only had some difficulties in climbing stairs and standing up from a squatting position. In the following years, the weakness progressed distally to the feet and he began to have troubles in running and long-distance walking. Four years later, the patient was referred to physicians for his difficulty in lifting his arms and aggravated trouble of walking. There were no behavioral symptoms. Neurological examination 5 years after onset revealed obvious atrophy of muscles of arms and lower limbs with fasciculations. Deep tendon reflexes were decreased in all limbs. Palm-chin reflexes and Babinski's sign was not elicited. EMG indicated the pattern of neurogenic changes in muscles of four limbs, such as abnormal spontaneous potentials, motor unit potentials of prolonged duration, increased amplitude, and reduced recruitment. Serum CK level was 298 IU/L (normal value 22–270 IU/L). The biopsy sample of the biceps muscle demonstrated grouped atrophic fibers with both type I and type II fibers. He scored 42/48 on ALSFRS-R and 28/30 on MoCA testing. He began to use wheelchair 7 years after onset. At the last follow-up visit at 102 months after onset, he was bedridden, but still independent for daily life activities, such as writing, dressing, and eating meals, without bulbar involvement. The ALSFRS-R score was 31/48 with an estimated progression rate of 0.2 score/month since symptom onset. His parents were in their 80s and healthy.

Patient 4 presented with weakness of the left leg at the age of 44 years and felt clumsy when climbing upstairs. After 2 years, he developed weakness of the right leg. Muscle atrophy with fasciculation in lower limbs was noticed. Serum CK level was normal. Serum alkaline phosphatase level was 1,532 U/L (normal value 50–135 IU/L). Shoulder, pelvic, and lumbar spine radiographs showed osteolysis, osteosclerosis, and cortical thickening. An isotope bone scan showed increased tracer uptake in the affected bones. He did not suffer from bone pain. He was diagnosed with PDB and treated with zoledronic acid injection, calcitriol, and calcium carbonate. The serum alkaline phosphatase level decreased obviously to about 200 U/L. He gradually developed weakness of both arms and had difficulties lifting arms. Neurological examination at 33 months after onset revealed obvious muscle atrophy of arms and legs with fasciculations. Muscle strength was decreased in the upper limbs (MRC 4/5 in distal limbs and MRC 3/5 in proximal limbs) and the lower limbs (MRC 3/5 in distal limbs and MRC 4/5 in proximal limbs). Deep tendon reflexes were decreased in all limbs. Babinski and Hoffman's signs were not elicited. He was cognitively normal, with a score of 28/30 on MoCA testing. He scored 42/48 on ALSFRS-R. EMG demonstrated chronic and active denervation of the upper and lower limbs, rectus abdominis, and sternocleidomastoid muscles. At the last phone follow-up at 45 months after onset, he could still walk independently for 1 km and do daily life activities, such as writing, dressing, eating meals, and driving, without bulbar involvement. The ALSFRS-R score was 39/48 with an estimated progression rate of 0.2 score/month since symptom onset. Both parents of patient 4 were healthy in their 70s.

Prevalence of VCP Mutations in Patients With ALS in Different Populations

We identified 44 studies screened VCP mutations in patients with FALS and/or SALS (**Table 2**). The frequency of VCP mutations is 0.08% (95% *CI* 0.00–1.26%) in patients with FALS and 0.02% (95% *CI* 0.00–0.15%) in patients with SALS in Chinese. The frequency of VCP mutations is 0.37% (95% *CI* 0.00–1.43%) in patients with FALS and 0.09% (95% *CI* 0.00–0.38%) in patients with SALS in Japanese. The frequency of VCP mutations is 0.28% (95% *CI* 0.12–0.52%) in patients with FALS and 0.08% (95% *CI* 0.03–0.15%) in patients with SALS in Caucasian populations. In pooled analysis, the frequency of VCP mutations is 0.28% (95% *CI* 0.12–0.50%) in patients with FALS and 0.06% (95% *CI* 0.02–0.12%) in patients with SALS (**Table 2**).

Literature Review of the Phenotype of ALS Patients With VCP Mutations

In addition, 46 VCP mutated ALS patients with detailed clinical features in previous research were identified. The clinical characteristics of these patients are summarized in **Table 3**. The mean age at onset was 50.29 ± 10.55 years, ranging from 24 to 68 years. Most of the VCP-related patients with ALS were Caucasian (76.1%, 35/46), only 9 cases were of Asian origin (19.6%, 9/46). Thirty-seven patients (80.4%) had a family history of ALS, FTD, dementia, parkinsonism, or PDB, including 31 patients (67.4%) who had a family history of ALS. Thirty-two (69.6%) patients claimed that the weakness started from limbs, and only six 13.0% patients developed a bulbar-onset. Co-occurrence of FTD, PDB, parkinsonism, myopathy, or psychiatric diseases was reported in 18 (39.1%, 18/46) ALS cases carrying VCP mutations.

DISCUSSION

In the present study, we reported the phenotype of four patients carrying the known VCP p.R155C mutation, such as two siblings from a familial ALS pedigree and a sporadic individual

TABLE 2 | Summary of studies screened VCP variants in patients with ALS.

	Race (origin)	FALS	FALS with *VCP* variants	SALS	SALS with *VCP* variants
Zou et al. (63)	Chinese	20	0	324	0
Liu et al. (11)	Chinese	20	0	234	0
Pang et al. (12)	Chinese	4	0	46	1
Tsai et al. (13)	Chinese	39	0	216	0
Zhang et al. (14)	Chinese	-	-	311	0
Liu et al. (15)	Chinese	24	0	-	-
Chen et al. (16)	Chinese	15	0	253	0
This study	Chinese	15	1	275	1
Hirano et al. (17)	Japanese	-	-	75	1
Nakamura et al. (18)	Japanese	39	1	469	0
Nishiyama et al. (19)	Japanese	111	0	-	-
Naruse et al. (20)	Japanese	89	1	410	1
Narain et al. (21)	Indian	5	0	149	0
Johnson et al. (5)	Caucasian	215	5	73	0
Williams et al. (22)	Caucasian	131	0	48	0
Abramzon et al. (23)	Caucasian	-	-	701	3
González-Pérez et al. (6)	Israeli-Arab	274	5	178	0
Koppers et al. (24)	Dutch	80	1	1,076	1
Miller et al. (25)	British	75	0	101	0
Tiloca et al. (26)	Italian	166	0	14	0
Kenna et al. (27)	Irish	50	0	389	1
Le Ber et al. (28)	French	-	-	26	0
Couthouis et al. (29)	American	-	-	242	0
McCluskey et al. (30)	American	20	1	-	-
Cady et al. (31)	American	42	1	349	0
Kwok et al. (32)	British	102	2	90	0
Krüger et al. (33)	German	6	0	74	1
Cooper-Knock et al. (34)	British	42	0	-	-
Gibson et al. (35)	American	-	-	87	0
McCann et al. (36)	Australian	212	0	-	-
Morgan et al. (37)	British	131	0	995	1
Türk et al. (38)	German	-	-	43	0
Dols-Icardo et al. (39)	Spainish	10	0	44	1
Lamp et al. (40)	Italian	58	0	210	0
Müller et al. (41)	German	301	1	-	-
Mehta et al. (42)	British	100	0	841	2
Tripolszki et al. (43)	Hungarian	3	0	104	0
Kotan et al. (44)	Turkish	10	1	45	0
Pensato et al. (45)	Italian	34	1	179	3
Ungaro et al. (46)	Italian	66	0	931	0
Yilmaz et al. (47)	German, Swedish	418	0	-	-
McCann et al. (48)	Australian	-	-	616	0
Nunes Gonçalves et al. (49)	Brazilian	93	1	-	-
Shepheard et al. (50)	British	7	0	93	0

ALS, amyotrophic lateral sclerosis; FALS, familial amyotrophic lateral sclerosis; SALS, sporadic amyotrophic lateral sclerosis.

from a Chinese ALS cohort of 275 sporadic and 15 familial ALS pedigrees.

Mutations in the VCP gene have previously been identified in Caucasian patients with ALS (5, 6, 24, 27, 45, 61, 62). Our meta-analysis showed that the presence of VCP mutations was 0.28% (95% *CI* 0.12–0.52%) in FALS and 0.08% (95% *CI* 0.03–0.15%) in patients with SALS in the Caucasian populations. The prevalence of VCP mutations in the Asian populations has not been well determined since the published ALS-VCP cases were mostly of Japanese origin (17, 52, 57, 58, 60). A VCP p.R487H mutation

TABLE 3 | Summary of previously published VCP-causing ALS cases with detailed records.

Base change	Amino acid change	Exon	Domain	Race (origin)	Family history	Gender	Age of onset (years)	Site of onset	Phenotype of ALS	Additional symptoms	Disease duration	Reference
c.179A>G	p.K60R	3	CDC48	Italian	No	F	<69	NA	Classic ALS	Cognitive impairment	NA	(45)
c.266G>A	p.R89Q	3	CDC48	Chinese	No	M	24	Limbs	Classic ALS	No	5 months	(7)
c.271A>T	p.N91Y	3	CDC48	Brazilian	Myopathy, FTD	M	36	Limbs	PMA	No	>4 years (alive)	(51)
c.277C>T	p.R93C	3	CDC48	Italian	ALS, PBD, AD	M	47	Lower limbs	Classic ALS	No	>13 years (alive)	(45)
c.293A>T	p.D98V	3	CDC48	Japanese	ALS	M	58	Limbs (proximal right leg, distal right arm)	Classic ALS	FTD	>10 years (alive)	(52)
c.340A>G	p.I114V	4	CDC48	Dutch	Dementia	F	52	Lower limbs (bilateral)	Classic ALS	No	>119 months (alive)	(24)
c.340A>G	p.I114V	4	CDC48	Caucasian	ALS	NA	45	Upper limbs (distal bilateral)	NA	No	27 months	(6)
NA	p.G128A	4	CDC48	Mixed Caucasian	ALS, PDB, PD, myopathy	M	48	NA	NA	No	NA	(53)
c.451A>G	p.I151V	5	CDC48	African-American	No	F	68	Lower limbs	Classic ALS	No	30 months (19 months to AV)	(54)
c.463C>T	p.R155C	5	CDC48	Italian	No	M	42	Limbs	Classic ALS	Myopathy	NA	(45)
c.463C>T	p.R155C	5	CDC48	Italian	ALS	F	29	Upper limb (left hand)	PMA	No	>11 years (alive)	(55)
c.463C>T	p.R155C	5	CDC48	Japanese	ALS, FTD	F	35	Upper limb (proximal right)	Classic ALS	No	5 months to AV, alive	(52)
c.463C>T	p.R155C	5	CDC48	American	ALS, FTD, myopathy, PBD	F	45	Lower limbs (proximal bilateral)	Classic ALS	Myopathy	3 years	(56)
c.464G>A	p.R155H	5	CDC48	Caucasian	ALS, PBD, IBM, parkinsonism, dementia,	NA	53	Upper limb (distal left)	NA	No	39 months	(5)
c.464G>A	p.R155H	5	CDC48	Caucasian	FTD, IBM, PBD, PD, psychiatric symptoms	NA	63	Limbs	Classic ALS	No	21 years	(6)
c.466G>T	p.G156C	5	CDC48	Japanese	ALS, psychiatric symptoms	M	51	Lower limbs	Classic ALS	No	>4 years (alive)	(57)
c.466G>T	p.G156C	5	CDC48	Japanese	ALS	F	34	Upper limbs	Classic ALS	Psychiatric symptoms	34 months (8 months to AV)	(57)
c.472A>G	p.M158V	5	CDC48	Japanese	No	M	36	Limbs (right)	NA	PDB	5 years (2 years to AV)	(58)
c.475C>G	p.R159G	5	CDC48	American	ALS, PDB, dementia	NA	53	Lower limbs	Classic ALS	Cognitive impairment	2 years to AV, alive	(5)
c.475C>G	p.R159G	5	CDC48	American	ALS, dementia	NA	46	Lower limbs	NA	PDB	5 years	(5)
c.475C>T	p.R159C	5	CDC48	American	No	F	68	Lower limbs	Classic ALS	No	>5 years (alive)	(23)

(Continued)

TABLE 3 | Continued

Base change	Amino acid change	Exon	Domain	Race (origin)	Family history	Gender	Age of onset (years)	Site of onset	Phenotype of ALS	Additional symptoms	Disease duration	Reference
c.475C>T	p.R159C	5	CDC48	Caucasian	ALS, PDB	NA	62	NA	Classic ALS	No	24 years	(6)
c.475C>T	p.R159C	5	CDC48	Caucasian	ALS	NA	57	Limbs	Classic ALS	PDB	NA	(6)
c.475C>T	p.R159C	5	CDC48	Caucasian	ALS, PDB	NA	53	NA	Classic ALS	No	16 years	(6)
c.475C>T	p.R159C	5	CDC48	Caucasian	ALS, PDB	NA	53	NA	Classic ALS	No	NA	(6)
c.476G>A	p.R159H	5	CDC48	Dutch	FTD, MS	F	59	Upper limbs (distal bilateral)	Classic ALS	No	23 months	(24)
c.476G>A	p.R159H	5	CDC48	Geek	FTD, dementia, myopathy	M	40s	NA	Classic ALS	No	>3 years (alive)	(59)
c.571C>G	p.R191G	5	Linker 1	Israeli-Arab	ALS, myopathy, parkinsonism	NA	50	Bulbar	Classic ALS	No	11 years	(6)
c.571C>G	p.R191G	5	Linker 1	Israeli-Arab	ALS, myopathy, parkinsonism	NA	42	Bulbar	Classic ALS	Myopathy	9 years to AV, alive	(6)
c.571C>G	p.R191G	5	Linker 1	Israeli-Arab	ALS, myopathy, parkinsonism	NA	<45	Bulbar	Classic ALS	Myopathy, parkinsonism	9 years	(6)
c.571C>G	p.R191G	5	Linker 1	Israeli-Arab	ALS, myopathy, parkinsonism	NA	<45	Bulbar	Classic ALS	Myopathy	NA	(6)
c.571C>G	p.R191G	5	Linker 1	Israeli-Arab	ALS, myopathy, parkinsonism	NA	<45	Bulbar	Classic ALS	Myopathy, parkinsonism	7 years to AV, alive	(6)
c.572G>A	p.R191Q	5	Linker 1	Italian	ALS, FTD/dementia, parkinsonism, PDB	NA	51	Upper limb (proximal right)	Classic ALS	No	29 months (11 months to AV)	(5)
c.572G>A	p.R191Q	5	Linker 1	Italian	ALS, FTD/dementia, parkinsonism, PDB	NA	53	Limbs (left)	Classic ALS	No	2 years to AV, alive	(5)
c.572G>A	p.R191Q	5	Linker 1	Italian	ALS, FTD/dementia, parkinsonism, PDB	NA	50	Right lower limb	Classic ALS	Cognitive impairment	>54 months (alive)	(5)
c.572G>A	p.R191Q	5	Linker 1	Italian	ALS, FTD, dementia, parkinsonism, PDB	NA	37	Lower limbs (distal bilateral)	Classic ALS	No	>4 years (alive)	(5)
c.572G>A	p.R191Q	5	Linker 1	Caucasian	ALS	M	42	Lower limb	Classic ALS	No	>12 years (alive)	(5)
c.572G>A	p.R191Q	5	Linker 1	Japanese	Demyelinating polyneuropathy, IBM	M	53	Lower limbs (distal bilateral)	Classic ALS	No	>1 years (alive)	(52)
c.572G>C	p.R191P	5	Linker 1	Turkish	ALS, FTD	F	60	Lower limb	Classic ALS	FTD	NA	(44)
c.572G>C	p.R191P	5	Linker 1	Turkish	ALS, FTD	F	48	NA	NA	No	NA	(44)
c.572G>C	p.R191P	5	Linker 1	Turkish	ALS, FTD	F	60	NA	Classic ALS	FTD	NA	(44)
c.1160A>C	p.N387T	10	D1	Caucasian	No	M	57	Lower limb	Classic ALS	No	>5 years (alive)	(23)
c.1460G>A	p.R487H	12	D2	Japanese	FTD, PD	M	61	Upper limbs (proximal bilateral)	PMA	Dementia	5 years to AV, alive	(17)
c.1460G>A	p.R487H	12	D2	Japanese	ALS	M	62	Left lower limb	Pyramidal ALS	FTD	78 months	(60)
c.1774G>A	p.D592N	14	D2	Caucasian	ALS	NA	52	Bulbar	Classic ALS	No	<1 year	(5)
c.1984C>T	p.R662C	14	D2	Caucasian	No	M	67	Lower limb	Classic ALS	No	>2 years (alive)	(23)

ALS, amyotrophic lateral sclerosis; AV, assisted ventilation; FTD, frontotemporal dementia; MS, multiple sclerosis; NA, not available; PD, Parkinson disease; PDB, Paget's disease of bone; IBM, inclusion body myopathy; IMV, invasive mechanical ventilation.

was identified in one out of 75 Japanese patients with SALS (1.3%) (17), and a p.R155C mutation was detected in one out of 39 Japanese FALS pedigrees (2.6%) but no VCP mutations were found in 469 SALS individuals (18). Our meta-analysis showed that the presence of VCP mutations was 0.37% (95% *CI* 0.00–1.43%) in FALS and 0.09% (95% *CI* 0.00–0.38%) in patients with SALS in Japanese (17–20). No VCP mutations have been discovered among Chinese patients with ALS (11, 13–16, 63) except Pang SY et al. reported a p.G157R mutation in one out of 46 SALS (12) and a recent study identified a p.R89Q mutation in one SALS case in a cohort of 27 unrelated young-onset patients with ALS (7). The higher frequency of VCP mutations in FALS (6.7%, 1/15) and SALS (0.4%, 1/275) in our study may be due to the small sample. Our meta-analysis showed that the frequency of VCP mutations is 0.08% (95% *CI* 0.00–1.26%) in FALS and 0.02% (95% *CI* 0.00–0.15%) in SALS in Chinese, which is lower than Japanese and Caucasian populations. The overall pooled mutation frequency of VCP mutations is 0.28% (95% *CI* 0.12–0.50%) in patients with FALS and 0.06% (95% *CI* 0.02–0.12%) in patients with SALS.

Valosin-containing protein p.R155C mutation was first associated with ALS in 2012 (6), before which it was found in patients with IBMPFD (64). The mutation was subsequently observed in two ALS cases without FTD or PBD from a large cohort study of 190 individuals carrying VCP variants (61) and another survey on 36 families with diverse VCP mutants (62). The onset age of the VCP p.R155C mutated patients in our study ranged from 42 to 51 years, consistent with the onset age of four VCP p.R155C-mutated ALS cases reported previously (29, 35, 42, 45, respectively) (**Table 3**). It seems that patients carrying VCP p.R155C mutations tend to have a young onset. The three patients carrying VCP p.R155C mutation in our study all had a limb-onset. Our system review revealed that 69.6% of VCP-related ALS had limb-onset ALS, and only patients carrying VCP p.R191G and p.D592N mutation had bulbar onset (**Table 3**). Patient 1 and 2 in our study demonstrated a phenotype of ALS while patient 3 had a phenotype of progressive muscular atrophy (PMA). They all presented with rather symmetrical proximal muscle weakness in the lower limbs at onset and subsequently progressed to distal lower limbs and upper limbs. Patient 4 manifested weakness in the left leg and developed a phenotype of PMA. Interestingly, two VCP p.R155C mutated patients with ALS reported previously also presented with symmetrical involvement of proximal muscles weakness of arms or legs (52, 56). The system review of previous studies showed that patients carrying VCP p.R487H and p.R159G mutation demonstrated the same phenotype (5, 17). The four p.R155C mutation carriers in our study demonstrated a slow progression (ΔALSFRS-R/month = 0.2 score/month) and two had a long survival duration of more than 102 months and 116 months, respectively. The grandfather (I-1) of the proband also exhibited signs of weakness in lower limbs and stayed bedridden for over 10 years before he died in his 70s. An Italian ALS patient with VCP p.R155C mutation reported by Battistini et al. also had a survival of more than 11 years (55). However, a 35-year-old female with p.R155C mutation progressed rapidly and received tracheotomy positive-pressure ventilation within 5 months after onset (52). It is interesting that in our study the father of proband who was supposed to carry the VCP p.R155C mutation had no symptom of muscle weakness and atrophy before he died of acute myocardial infarction at the age of 72 years, indicated incomplete penetrance of VCP p.R155C mutation. Phenotypic variability in VCP p.R155C mutated ALS pedigree has been reported. In an Italian family, the proband with harboring VCP p.R155C mutation developed young-onset ALS with diffuse severe weakness and wasting in the limbs and rimmed vacuoles in muscle biopsy, while his mother and maternal aunt suffered from mild symptoms limited to lower limbs (45). Our system review showed that ALS patients with some VCP mutations (p.R93C, p.D98V, p.I114V, p.R155C, p.R155H, p.R159C, p.R191G, and p.R191Q) had a relatively slow progression and survival of more than 10 years (**Table 3**), which is consistent with the phenotype of our patients. However, some ALS patients with VCP mutations (p.R89Q, p.I114V, p.I151V, p.R155C, p.G156C, p.R159H, p.R191Q, and p.D592N) developed a rapid progression and had a survival of fewer than 3 years (**Table 3**). Phenotype variability associated with different or the same VCP mutation suggests the possible role of modifying genes and/or environmental factors. Extensive genetic studies in different populations to identify more ALS patients with VCP mutations may provide more insight into the genotype-phenotype correlations and the diversity of clinical phenotypes of VCP mutations.

Patient 4 carrying VCP p.R155C mutation developed a phenotype of PDB in addition to PMA. A VCP p.G97E mutation was reported in a Chinese family with IBMPFD without ALS (65). Patient 4 in our study was the first VCP mutation carrier manifesting PDB in addition to ALS in the Chinese population. The co-occurrence of FTD, PDB, parkinsonism, myopathy, or psychiatric diseases was commonly seen in the ALS cases carrying VCP mutations (**Table 3**). The co-existence of FTD or cognitive impairment was found in patients with seven VCP mutations (p.K60R, p.D98V, p.R155C, p.R159G, p.R191Q, p.R191P, and p.R487H) (5, 17, 44, 45, 52, 56, 60), while PDB was diagnosed in ALS patients with six VCP mutations (p.R93C, p.G128A, p.R155C, p.M158V, p.R159G, and p.R159C) (5, 6, 45, 53, 56, 58). Co-occurrence of myopathy was reported in ALS patients with VCP p.R155C and p.R191G mutations (6, 45, 56), and psychiatric disorders were found in patients with p.G156C and p.R159G mutations (5, 57). Parkinsonism is presented in familial ALS cases with VCP p.R191G mutation (6). Increasing evidence has shown that VCP-related disease may be a multisystem proteinopathy that has a wide clinical spectrum of IBM, FTD, PDB, and parkinsonism apart from ALS (66–68). The phenotypic diversity of the same VCP mutation may indicate the possible role of modifying genes and/or environmental factors.

Except for the hexanucleotide expansions and single base-pair substitutions in the 5' UTR or 3' UTR region of VCP predicted as pathogenic without definite experimental evidence (32), 26 mutations in 17 exons of the VCP gene have been identified in patients with ALS (**Figure 1C**). All mutations are heterozygous missense mutations. Residues of R155, R159, and R191 are three hot spots of VCP mutations of patients with ALS. VCP is divided

into 4 domains, such as one N-terminal CDC48 region, two AAA ATPase domains (D1 and D2), and one C-terminal domain. VCP mutations are predominantly located within the N-terminal ubiquitin-binding domain (69.2%, 18/26), indicating that the malfunction of poly-ubiquitinated protein degradation may be the major pathogenesis of ALS (2). Within the ring-shaped VCP hexamer, the N-terminal domain serves as an indispensable binding region for interaction with target co-factor molecules (2). When pathogenic mutations occur in the N-domain of VCP protein, such as p.R155C, p.R159H, p.R95G, p.G97E, and p.A232E, the stress response is impaired resulting in the incorrect translocation of this hexameric ATPase and assembly disorder (69). Besides the aberrant aggregation of transactive response DNA-binding protein of 43 kDa (TDP-43) (5, 70), the nuclear-to-cytoplasmic mislocalization of fused in sarcoma (FUS) protein in motor neurons has been identified as another pathogenic feature of VCP-mutated ALS, which was ascribed to the increased intron retention (IR) in splicing factor proline and glutamine rich (SFPQ) transcripts (71, 72). Using ALS patient-specific induced pluripotent stem cell (iPSC) models, Patani et al. further unveiled that the four abnormal accumulated sequence-specific intron retention transcripts (IRTs) in VCP mutations included SFPQ, OGT, TUSC3, and DDX39, and their binding affinity to RNA binding proteins (RBPs) could be the key attributes for RBP localization (73, 74). In future, further experiments on pathomechanism of VCP-mutated ALS should be conducted on transgenic animal models as well as patient-specific iPSC models.

In summary, we reported the first VCP mutation carrier manifesting ALS with PDB of ALS in the Chinese population. Our findings expand the phenotypic spectrum of VCP mutations in Chinese patients with ALS and suggest that ALS patients with VCP p.R155C mutation tend to present with relatively young onset, symmetrical involvement of proximal muscles weakness of arms or legs, and then progressed to distal muscles of limbs.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, sra/PRJNA791140.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Fujian Medical Union Hospital and Henan Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Z-YZ and S-YF designed and conceived the study. Z-YZ, S-YF, and HL performed the analysis of mutations in all the patients. HL wrote the manuscript. Z-YZ critically revised the manuscript. All remaining authors participated in the analysis of data, discussion of the final manuscript.

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Tau Ubiquitination in Alzheimer's Disease

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Paired helical filaments (PHFs) from the Alzheimer's disease (AD) brain are highly ubiquitinated and ubiquitination likely plays a vital role in tau filament formation. Whether tau ubiquitination is the causality or consequence of the disease in AD remains elusive. The following questions are worth considering: What does the extent of tau ubiquitination contribute to tau pathology in AD? Does tau ubiquitination influence aggregation or spreading during disease progression? In addition, tau is polyubiquitinated in nerve growth factor-induced PC12 cells and participates in mitogen-activated protein kinase signaling, in addition to its microtubule stabilization function. Therefore, ubiquitination possibly mediates tau signaling under physiological conditions, but tau aggregation in the pathobiology of AD. Here, we review the advancements in tau ubiquitination and the potential therapeutic effects of targeting tau ubiquitination to alleviate tau pathology in AD.

Keywords: PHF, Alzheimer's disease, tau, NGF, ubiquitination

INTRODUCTION

Ubiquitin-positive inclusions are characteristic of neurodegenerative diseases, such as Alzheimer's disease (AD) and frontal temporal lobe degeneration (1–3). Neurofibrillary tangles, mainly consisting of hyperphosphorylated tau, are also ubiquitin and p62 positive in AD (4, 5). In fact, ubiquitin is elevated by many folds in the AD brain determined by immunoassay (1), and the ubiquitination level increases at ∼80% ubiquitylated sites from the altered 800 ubiquitination sites by label-free mass spectrometry (MS)-based proteomic analysis (6). Among the ubiquitination proteins, the microtubule-associated protein tau has the highest number of sites of ubiquitination per protein in AD (6). In addition, the data from cryoelectron microscopy (EM) and MS of tau filaments from AD and corticobasal degeneration brain further demonstrate ubiquitination of tau in neurofibrillary tangles, and this posttranslational modification might play a structural role in its fibrillation and fibril heterogeneity (7–9). This review aimed to conclude the influence of tau ubiquitination in the pathobiology of AD and provide valuable cues for future studies.

TYPE OF TAU UBIQUITINATION IN AD

The 76-amino acid protein ubiquitin can be transferred to lysine with subjects through the three enzyme cascade steps: ubiquitin activation, conjugation, and ligation by E1, E2, and E3 enzymes in succession (10). The substrate can be either modified by a monomer or a polyubiquitin chain depending on E2s, whereas the substrate specificity is determined by E3 ligases (11).

In addition to monoubiquitylation, a polyubiquitin chain occurs when another ubiquitin is conjugated to any of the seven lysines or N-terminus on the first ubiquitin by its C-terminal GG amino acid (12). Ubiquitin modification and its signal for cell output and degradation are reviewed in detail (10–14). In this review, these were not specified, and this review only focused on tau ubiquitination in AD.

The microtubule-associated protein tau has 44 lysine residues on the longest 441 isoforms, most of which are located on the proline-rich domain and microtubule-binding domain repeat (MTBR) (15). Tyrosine kinase family proteins, such as fyn and c-src, bind to tau with the PXXP motif in the proline-rich domain and execute its function (16, 17), whereas tau promotes microtubule stabilization through the MTBR domain (18). The abundant lysine and arginine make full-length tau pI = 8.24, and tau's repeat domain (termed K18) pI = 9.73 (15). Furthermore, 28 tau ubiquitylation sites detected in human AD brain samples underlie the tau to become the highest number of increased ubiquitination sites per protein, as discussed above (6). Among these sites, K257, K259, K267, K274, K281, K290, K321, K343, K353, K375, and K385 are ubiquitinated by E3 ligase Chip (**Figure 1**) (7). The most frequently reported sites are Lys254, Lys257, Lys311, Lys317, and Lys353 (6, 8, 19, 20).

To date, K48-, K63-, K6-, K11-, and M1-linked polyubiquitin chains have been verified in tau of paired helical filament (PHF), although the majority of ubiquitination in PHF is in monoubiquitinated form (19–21). K48-linked polyubiquitination is the most common form of tau poly-Ub and is hypothesized to mediate protein degradation by the proteasome system (19). K63-linked poly-Ub of proteins can serve multiple functions for various proteins, including promoting insoluble inclusion formation (22), signal for autophagy lysosome (13), endocytosis (23), and DNA repair (24). A part of PHF is K63-linked poly Ub-positive, as detected in the AD brain (25). One study reported that to a lesser extent K6/11 poly-Ub existed in PHF by tandem mass spectrometry assay, and K6-linked poly-Ub may inhibit proteasome activity (20). M1-linked linear poly-Ub, which is involved in neuronal cell death, appears after K48-linked poly-Ub of PHF (21, 26). A few studies revealed that tau mono-Ub impaired its microtubule binding (27) and that the N-terminal tau mono-Ub could impede its aggregation by cooperating with the proteasome system (28).

REGULATION OF TAU UBIQUITINATION IN AD

The identification of the types of tau ubiquitination is accompanied by the recognition of the E1, E2, and E3 enzymes involved in tau ubiquitination. Regarding mono-Ub, axotrophin/MARCH7 containing a RING-variant domain could monoubiquitinate tau combined with E2 UbcH5 and impair its microtubule binding (29). A recent study reported that E2 Ube2W attached mono-Ub to the αN-terminus of tau by recognizing backbone atoms of disordered N-termini (30). *In vivo*, CHIP modifies tau through both K48- and K63-linked polyubiquitination (31), and tau is also a K63-polyubiquitinated substrate of TRAF6/UbcH7 (32). Furthermore, the ubiquitin elongation enzyme UBE4B has been reported to promote tau polyubiquitination sufficiently by cooperating with CHIP, thereby promoting tau degradation (33).

In addition to identifying E3 ligase on tau ubiquitination, other inducers, such as nerve growth factor (NGF), can promote tau ubiquitination through unknown mechanisms. NGF maintains neuronal survival and differentiation in PC12 through its receptor TrkA with the elevation (>2-fold) of ubiquitin conjugate levels; a similar result could be mimicked by a proteasome inhibitor (34). NGF also stimulates tau ubiquitination through K63-linked poly-Ub in neuronal cell differentiation (25), which implies the possibility of the downregulation of the NGF signaling pathway in AD pathogenesis by impairing normal tau ubiquitination, as the decrease of NGF in the cortex and hippocampus mediates cholinergic neuron degeneration in the basal forebrain at disease onset.

ROLE OF UBIQUITINATION IN TAU TURNOVER

Six isoforms of tau by alternative slicing are mainly expressed in the central nervous system (CNS) (35). CNS tau is a long-lived and natively disordered protein, which has a half-life of ∼23 days. Under physiological conditions, tau is soluble and has a hairpin structure in which the MTBR is buried under the inner layers of the C-terminus and the outer layers of the N-terminus, as detected by fluorescence resonance energy transfer (36). An average of 2–3 mol of phosphate per mole of protein in normal people and 6–8 mol of phosphate per mole of protein in the AD brain indicates that abnormal hyperphosphorylation of tau plays an important role in the pathobiology of AD (37–39). Several studies have already reported how hyperphosphorylation of tau leads to neurotoxicity and epigenetic risk factors, such as trauma, sleeplessness, and less exercise, influencing tau phosphorylation and cognitive function (40). Thus, tau phosphorylation is widely used as a biomarker in cell and animal models in AD studies. Tau phosphorylation at threonine 181 and 217 in the cerebrospinal fluid or plasma have shown their potential as a biomarker for predicting the extent of dementia (41, 42). AT8 staining is considered the gold standard for detecting pretangles in immunohistochemistry (IHC), as it is applied by BraaK (43).

Phosphorylation of tau could change its conformation and open its hairpin structure, resulting in the formation of tau oligomers despite the exact mechanisms of oligomer formation being still elusive. Many factors can affect this process, such as extending its N-terminus or C-terminus to protease, resulting in truncation and accelerating tau aggregation, or being recruited to stress granules with its abundant lysine in the proline-rich (PR) and MTBR domains under cell stress (15, 44–47). In 1989–1991, it was reported that ubiquitin levels increased many folds in the AD brain, and the accumulation of abnormally phosphorylated tau preceded the formation and ubiquitination of neurofibrillary tangle (NFT) (1, 48, 49). About 2 years later,

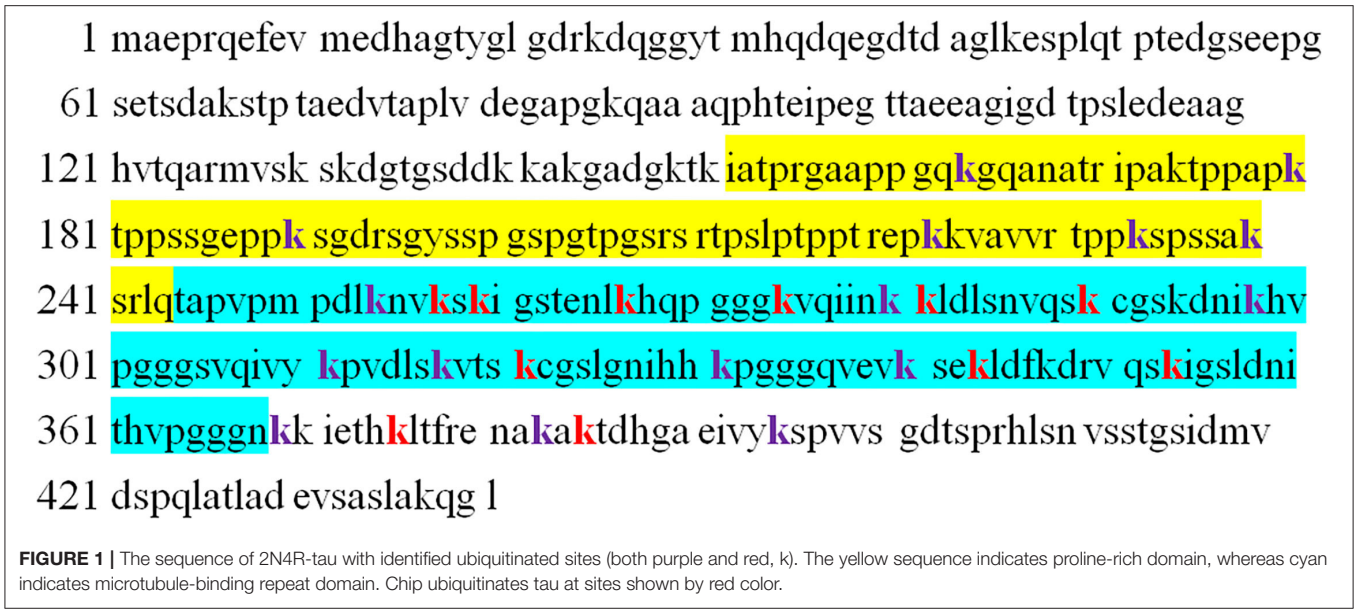

FIGURE 1 | The sequence of 2N4R-tau with identified ubiquitinated sites (both purple and red, k). The yellow sequence indicates proline-rich domain, whereas cyan indicates microtubule-binding repeat domain. Chip ubiquitinates tau at sites shown by red color.

Yasuo Ihara et al. purified high-molecular weight (HMW) Ub (–) PHF and ubiquitin (+) PHF from PHF tau and found that the amino-terminal portion of tau was truncated in Ub (–) PHF and to a greater extent in HMW ubiquitin (+) PHF (19). A more detailed study was conducted several years later, using tau oligomer-specific antibody T22 in combination with pT231, AT8, and ubiquitin antibodies. Kayed et al. proposed a more specific sequence of tangle formation with minor revision of previous results: tau phosphorylation at the T231 site (stage 0); tau oligomer initiation and formation with pT231 (stage 1) and intraneuronal NFT (iNFT) containing mixed oligomer, protofilament, and filament (stage 2); and neuronal death and the formation of ghost tangle with T22 negativity and pT231 positivity, referred to as extraneuronal NFT (eNFT) (stage 3). Besides the AT8 property of the staining pretangle, most eNFTs are AT8 positive. By comparing the time sequence of ubiquitination and tau oligomerization, they suggest that ubiquitination does not appear at the initial stage of tau oligomerization, but it occurs on iNFT (stage 2) followed by high ubiquitination on eNFT (50). The above studies suggest that abnormal phosphorylation likely mediates tau oligomerization (51). As the soluble tau oligomer grows and is modified by truncation and ubiquitination, it gradually matures to fibrillary tangles and is insoluble with tangle formation. This transformation suggests that ubiquitination probably promotes insoluble filament formation from soluble tau aggregates (8, 52).

To better understand the effect of ubiquitination on tau aggregation, Kah-Leong Lim explored the contribution of K48- and K63-linked poly-Ub and monoubiquitination to the biogenesis of inclusions in cell models and found that K63-linked poly-Ub usually promoted inclusion formation, such as tau and sod1 aggregates and their clearance by autophagy (22). In fact, CHIP-mediated K48-/K63-linked poly-Ub promotes insoluble tau formation, whereas HSP70 suppresses it (31). Moreover, CHIP knockout mice show accumulation of nonaggregated, ubiquitin-negative, and hyperphosphorylated tau, demonstrating that CHIP mediates ubiquitin-dependent tau degradation and poly-Ub of tau by CHIP accelerating the formation of insoluble filaments (53). CHIP is an E3 ligase of phosphorylated tau. CHIP mediates tau ubiquitination at K267, K290, K343, and K353 sites, but additional K257, K259, K274, K281, K321, K375, and K385 are ubiquitinated by CHIP after tau is phosphorylated by glycogen synthase kinase-3β. Prolonged phosphorylated tau ubiquitination promotes tau aggregation (7). In another study, traf6-mediated K63-linked poly-Ub also showed a similar effect on tau aggregation (32).

Contrary to these studies, Munari et al. recently studied the aggregation rate of K18 and Ub-K18 *in vitro* by applying semisynthetic and enzyme-mediated conjugate methods (27). They used CHIP/UBC13 rather than E2 UBCH5 to perform K18 (fragment of 4R-tau) ubiquitination in the tube, which resulted in tau mono-Ub rather than poly-Ub. Unexpectedly, these modifications make K18 lose the ability to convert into amyloid fibrillary structures. Meanwhile, conjugating the mono-Ub to a specific site in K18 by the semisynthetic method would slow down the aggregation but permit the aggregation formation at Lys254 and mostly at Lys353. Lys 311 ubiquitination might follow the formation of NFT and determine fibril subtypes because it is located within the core region of tau for the formation of NFT, which will hinder the aggregation (27).

The N-terminal connected mono-Ub of K18 by UBE2W also slows down the K18 aggregated kinetics and targets oligomers formed by N-mono-Ub-modified K18 to proteasomal degradation compared to unmodified K18 (28). These studies suggest that ubiquitination influences tau aggregation *in vitro*. However, it does not consider which ubiquitination usually occurs later than tau phosphorylation and oligomerization, as discussed above, in pathological conditions, although tau monomer needs ubiquitination to be degraded by proteasomes in physiological status.

Proteasomes have recently been shown to fragment tau filaments into oligomers *in vitro* and degrade oligomers formed from N-terminal mono-Ub-linked K18 (54). Regardless of whether CHIP or Traf6 mediates tau ubiquitination, both have been demonstrated to promote tau degradation by the proteasome. In contrast, tau oligomers can impair proteasomes and decrease proteasome activity in AD brains (55). Therefore, the following should be taken into consideration: (1) Elevation of ubiquitin several folds in the AD brain and ubiquitin-positive inclusions being common in neurodegeneration; (2) the spatiotemporal order of tau phosphorylation, oligomerization, and ubiquitination during the formation of PHF; (3) loss of proteasome activity in the AD brain (56); and (4) the neurotoxicity of oligomer tau rather than PHF (57, 58). This can outline the following vicious circle model in cells to explain the roles of ubiquitination in the regulation of tau degradation and aggregation: (1) protein folding-refolding machinery (CHIP/HSP70/90) supervises the folding status of tau (normal tau stage); (2) once tau is abnormally phosphorylated with conformational changes and forms oligomers, a protein quality control system will observe these changes and initiate ubiquitination of the misfolded protein, which promotes insoluble tau formation and its degradation by the proteasome (oligomer tau stage); and (3) with consistent oligomer tau stages, the proteasome is overloaded and cracked; however, without the normal function of the proteasome, these insoluble aggregates accumulate over time and mature into filaments with further ubiquitination, phosphorylation, and truncation (Ub + PHF) (NFT stage).

IS TAU UBIQUITINATION BENEFICIAL TO NEURON SURVIVAL IN AD?

Despite evidence suggesting that tau ubiquitination is involved in tau pathology, including tau mislocated to dendrites or promoting tau aggregation (7, 59), there is no direct evidence showing that tau ubiquitination is neurotoxic. However, as the primary function of ubiquitination is to mediate protein degradation, tau ubiquitination is likely nontoxic. Hyperphosphorylated soluble and oligomer tau is hypothesized to have the highest seeding activity and is the most toxic tau species among tau monomers, oligomers, and multimers (58, 60–63). As discussed above, ubiquitination increases tau insolubility and occurs at a relatively late stage during the formation of NFTs. Thus, soluble and toxic tau oligomers are transformed into insoluble and less toxic NFTs after hyperubiquitination. In fact, neurons bearing NFTs can survive for many years (64, 65). Soluble tau, rather than insoluble NFTs, results in neuronal loss and cognitive dysfunction in a model of tauopathy (57). Based on this evidence, ubiquitination likely protects neurons from the toxicity of oligomer tau by promoting insoluble NFT formation, which is consistent with the concept that polyubiquitination promotes tau aggregation. In contrast, the prion-like activity of pathologic tau decreases with longevity, despite an increase in insoluble tau in AD, and the seeding activity of oligomer tau is higher than that of sarkosyl-insoluble tau. Thus, we can hypothesize that ubiquitination will decrease the seeding and toxic activity of oligomer tau by promoting insoluble NFT formation, but this needs to be proven by experiments. Under physiological conditions, the tau monomer or oligomer is ubiquitinated and mediated to the proteasome for degradation. However, as AD progresses, proteasome impairment results in the accumulation of ubiquitinated proteins. The dysfunction of this process might contribute to the formation of NFTs and result in neurofibrillary neurodegeneration (**Figure 2**).

ALZHEIMER'S DISEASE THERAPEUTIC APPROACHES BASED ON TAU UBIQUITINATION

As discussed above, ubiquitination is good for tau degradation or promotes insoluble and less-toxic aggregate formation from soluble toxic oligomers under physiological or pathological conditions. Therefore, targeting tau ubiquitination should be an effective approach for the treatment of AD. In fact, the benefits of tau ubiquitination are supported by the fact that upregulation of tau ubiquitination through biochemical methods can reduce tau levels and improve impaired memory in an AD mouse model *in vivo*. Using a chimera with tau binding domain and E3 ligase-binding moieties, TH006 can specifically induce endogenous tau degradation by promoting tau polyubiquitination (66). A similar chimera, C004019, with the same routine has similar effects and improves cognitive function in an AD mouse model (67). In addition, mislocated tau in the postsynaptic fraction is hyperphosphorylated and ubiquitinated, and tau from the postsynaptic fraction is a seeding component. Promoting tau degradation in this area by elevating proteasome activity by stimulating the PAC1 receptor-mediated cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway has been shown to improve impaired cognitive performance in rTg4510 mice (67). Another direct evidence is from the Drosophila transgenic model. Overexpression of UBE4B, an E4 ubiquitin elongation enzyme, promotes tau ubiquitination and degradation and alleviates eye neurodegeneration, which is blocked by knockout of the E3 ligase CHIP. Furthermore, this enzyme can reduce tau oligomer levels in a tau transgenic model (33). Based on this evidence, we can conclude that promoting tau ubiquitination or elevating proteasome activity promotes tau degradation and improves tau-induced neurodegeneration and cognitive dysfunction *in vivo*, despite the fact that hyperpolyubiquitination promotes tau aggregation *in vitro*. Therefore, promoting tau ubiquitination along with elevated proteasome activity might provide an effective avenue for AD treatment by reducing tau protein levels.

CONCLUSION AND REMARKS

Ubiquitination can induce ubiquitin-positive and ubiquitin-insoluble tau formation, which could mediate protein degradation. Polyubiquitination protects cells from the toxicity of the soluble oligomer tau. However, these ubiquitin-positive and insoluble tau aggregates accumulate in cells if the degradation

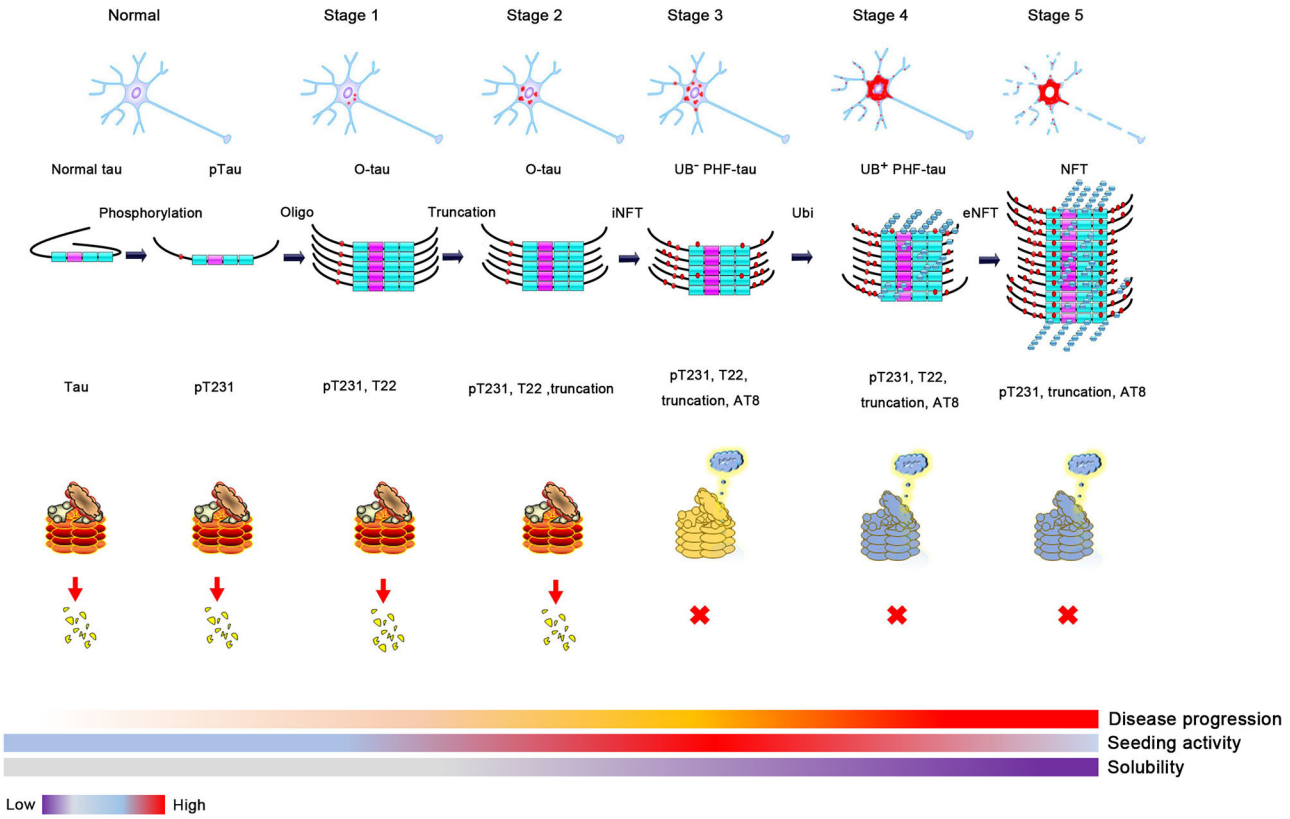

FIGURE 2 | The proposed process of tau aggregation. Phosphorylation changes tau conformation and induces oligomer tau aggregation. Soluble oligomer tau has higher toxicity and seeding activity. Proteases, such as caspase 3, calpain, or legumain, can cleavage tau and result in tau truncation when the cell is toxic. Monomer and oligomer tau can be ubiquitinated and degraded by the proteasome. However, ubiquitin-positive tau aggregation will be accumulated when the activity of proteasome is decreased (high activity, red; medial activity, yellow; and low activity, blue), which will decrease the seeding activity and solubility of tau aggregation and finally lead to the formation of neurofibrillary tangles formation.

system is damaged under disease conditions. Ubiquitination of tau is good for cell survival, as many studies aimed to induce tau ubiquitination artificially, having beneficial effects on improving abnormal behavior in an AD mouse model. In addition, the genetic upregulation of E3 or E4 ubiquitin ligases is also useful. Among them, CHIP is the most well-studied E3 ligase for tau ubiquitination. CHIP mainly ubiquitinates phosphorylated tau and promotes the formation and degradation of insoluble tau. Deletion of CHIP in P301L mice led to soluble and phosphorylated tau accumulation. Furthermore, the sites in tau ubiquitinated by CHIP have recently been identified *in vitro* (7). These studies strongly suggest that ubiquitination promotes NFT formation. In other words, NFT formation might be a consequence of tau ubiquitination without being efficiently degraded by the proteasome. Thus, promoting proteasome activity degrades these ubiquitin-positive tau aggregates. In contrast, reducing tau ubiquitination by inactivating E3 ligase results in soluble tau accumulation, which is a disaster for cell survival. Because HMW tau oligomers in the soluble fraction have higher seeding activity and are the most toxic, it is worthwhile to study the effect of ubiquitination on oligomer tau seeding activity and neurotoxic ability. As phosphorylation or acetylation of tau has been widely explored, the role of ubiquitination in tau-mediated neurodegeneration is believed to be the next hot topic in the future.

AUTHOR CONTRIBUTIONS

XW framed and reviewed the manuscript. LL and YJ organized the literature and wrote the manuscript. J-ZW and RL analyzed and discussed the manuscript. All the authors read and approved the final version of the manuscript.

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BRIEF RESEARCH REPORT
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Good Performance of the Chinese Version of Mini Social Cognition and Emotional Assessment in the Early Diagnosis of Behavioral Variant Frontotemporal Dementia

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Social cognition impairment has been recognized as an early and characteristic change in behavioral variant frontotemporal dementia (bvFTD). The Mini Social Cognition and Emotional Assessment (mini-SEA) is a clinical tool to rapidly evaluate social cognition. In this study, we explored the diagnostic value of social cognition by assessing the Chinese version of the mini-SEA and other standard neuropsychological tests in 22 patients with mild bvFTD, 26 patients with mild Alzheimer's disease (AD), including mild cognitive impairment (MCI) and mild dementia, and 30 control subjects. The discriminatory powers of these tests were evaluated and compared using the receiver operating characteristic curve (ROC). The mini-SEA scores of the bvFTD patients were significantly lower than those of the controls ($Z = -6.850$, adjusted $P < 0.001$) and AD patients ($Z = -3.737$, adjusted $P = 0.001$). ROC analysis showed that the mini-SEA had a high discriminatory power for differentiating bvFTD from the controls, with an area under the curve (AUC) value of 0.989 (95% $CI = 0.905$-1.000, $P < 0.001$). The AUC value of the mini-SEA for differentiating bvFTD from AD was 0.899 (95% $CI = 0.777$-0.967, $P < 0.001$), higher than that of the Auditory Verbal Learning Test Delayed Recall (AUC = 0.793), Boston Naming Test (AUC = 0.685) or Frontal Assessment Battery (AUC = 0.691). The Chinese version of mini-SEA is a good clinical tool for the early diagnosis of bvFTD, and has a high sensitivity and specificity to discriminate bvFTD from AD.

Keywords: mini-SEA, social cognition, behavioral variant frontotemporal dementia, Alzheimer's disease, early diagnosis

INTRODUCTION

Behavioral variant frontotemporal dementia (bvFTD) is a pathologically and genetically heterogeneous neurodegenerative disorder characterized by progressive behavioral abnormalities, personality changes and impaired social interaction (1). It is a leading cause of early-onset neurodegenerative dementia along with Alzheimer's disease (AD) (2). Due to the absence of definitive biomarkers, bvFTD is difficult to identify accurately in the early stages and may be underdiagnosed or misdiagnosed as AD, depression, or other psychiatric disorders (3). In 2011,

the International Behavioral Variant Frontotemporal Dementia Criteria Consortium (FTDC) revised the diagnostic criteria of bvFTD, and classified the diagnosis into three levels: possible, probable and definite bvFTD (4). The diagnostic criteria are sensitive and practical, but accurate diagnosis of bvFTD remains to be improved (5). Many research showed that the criteria on the neuropsychological profile (F: executive deficits with relative sparing of memory) might not be optimal. The application of traditional executive function tests in the early and differential diagnosis of bvFTD has yielded inconsistent results (6, 7). Recent studies also reported that large impairment in memory also occurred in patients with bvFTD and should not be regarded as an exclusion criterion (8). More and more researchers are turning their attention to social cognition testing in order to improve the criteria of bvFTD.

Social cognition is the ability to perceive and interpret information about other people and social situations, and plays a key role in social behaviors and interpersonal communication. It is usually thought to include theory of mind (ToM), emotion recognition, empathy, social knowledge and insight (9). The neural substrate for social cognition is medial prefrontal cortex (mPFC), orbitofrontal cortex, anterior cingulate cortex, insula and amygdala, etc. (10), which are obviously affected early in bvFTD (11). Therefore, social cognition impairment has been thought to be an early and characteristic change in bvFTD, and many cognitive tests for social cognition are being actively developed and applied in the western population (9). Social cognition is greatly influenced by culture (12). Anthropologist and psychologists have long known that individuals raised in different cultures, East and West, have different understandings of themselves and their relationships with others, referred as the "collectivism" and "individualism" (13). Both emotion recognition and expression are related to culture, and facial expressions are easier to recognize by members of the same ethnic or national group (14). Moreover, neural correlates of ToM may also differ across cultures (15), and medial prefrontal cortex, the key structure of the social neural network, is suggested to be employed in a culture-dependent manner (13). Therefore, it is critical to develop culturally appropriate clinical tools to evaluate social cognition.

The Mini Social Cognition and Emotional Assessment (mini-SEA) is a tool to rapidly evaluate ToM and emotion recognition clinically (16). It has been considered as a promising diagnosis tool for bvFTD in the early stages and reported to have a good sensitivity and specificity to distinguish bvFTD from AD (17). However, the mini-SEA, developed in the western cultural context, may not suitable for Chinese bvFTD patients, given the striking cultural differences in social cognition. The adaptation of the mini-SEA will help us to investigate social cognition in Chinese population, and verify whether clinical features of bvFTD are comparable across different ethnicities. In this study, we used the Chinese version of mini-SEA and other standard neuropsychological tests in patients with mild bvFTD and AD, and aim to (1) assess the social cognition profile and other cognition domains (executive function, episodic memory, language, etc.); (2) explore the value of the Chinese version of mini-SEA for the early diagnosis of bvFTD and its ability to discriminate bvFTD from AD.

MATERIALS AND METHODS

Participants

Seventy-eight participants including 22 patients with mild bvFTD, 26 patients with mild AD and 30 control subjects were recruited from January 2018 to December 2019. All of the patients (bvFTD and AD) were enrolled from the memory clinic of Xuanwu Hospital and each patient and his/her guardian received a semi-structured interview collecting detailed demographic data and medical history. All patients underwent a standard physical and neurological examination, a neuropsychological test battery, blood tests (blood routine, liver and kidney functions, serum levels of electrolyte, ammonia, homocysteine, folic acid, vitamin B12, and thyroid hormone, syphilis and AIDS antibody) and brain conventional magnetic resonance imaging (MRI) scan. 18F-Fluorodeoxyglucose positron emission tomography (18F-FDG PET) scans were performed for some bvFTD and AD patients to reveal the frontotemporal or temporoparietal hypoperfusion. To further clarify the diagnosis, some patients also underwent cerebrospinal fluid (CSF) examination to test p-Tau and Aβ-42 level, and/or Amyloid-PET, and/or related genetic testing. The final diagnosis of each patient was approved by two clinical experts on dementia. In order to improve the diagnostic accuracy, all patients were followed up for 6–12 months.

The patients with bvFTD met the revised diagnostic criteria for the probable or definite bvFTD proposed by FTDC in 2011 (4). All the patients had progressive deterioration of behavioral and/or cognition with the Mini-Mental State Examination (MMSE) scored >20/30, Clinical Dementia Rating (CDR) scored 0.5 or 1, meeting the requirements for the mild stage of bvFTD established in this study. Brain MRI and/or FDG-PET showed frontotemporal atrophy and/or hypoperfusion. Some patients underwent the lumbar puncture ($n = 12$) and amyloid-PET ($n = 3$) to excluded AD and other types of dementia. Genetic testing was performed in 11 patients with bvFTD. Two of them carried *MAPT* mutation (P513A and P301L) and one carried *GRN* mutation (S106R). Fourteen patients with bvFTD did not take any drugs before enrollment. Five patients have treated with serotonin reuptake inhibitors, two patients with Donepezil, and one patient has taken olanzapine.

The patients with AD met the revised diagnostic criteria for mild cognitive impairment (MCI) due to AD ($n = 15$) or probable AD dementia ($n = 11$) proposed by National Institute on Aging and Alzheimer's association (NIA-AA) in 2011 (18, 19). All the AD patients, including MCI or mild dementia (MD), had progressive episodic memory loss with MMSE scored >20/30 and CDR scored 0.5 or 1. Brain MRI and/or FDG-PET showed hippocampal atrophy and/or medial temporal hypoperfusion. All the patients with MCI due to AD had the evidence of amyloid deposition (elevated CSF p-Tau/Aβ-42 and/or positive amyloid-PET). None of the AD patients received any antidepressant, antipsychotic, or antidementia drugs before enrollment.

The patients were excluded if they met one of the following items: (1) vascular cognitive impairment supported by medical history and/or MRI findings; (2) clinically predominant aphasia; (2) motor neuron disease; (3) inflammatory, metabolic and other related disorders that cause cognitive impairment; (4) severe visual and auditory impairment interfering the cognitive assessment.

The gender- and education-matched healthy control subjects were recruited from the patients' spouse or healthy elderly at physical examination center of Xuanwu Hospital. The controls were included in the study according to the following criteria: (1) MMSE scored $\geq$ 27/30, CDR = 0 and Frontal Assessment Battery (FAB) $\geq$ 16/18; (2) No memory complaints or behavior problems or cognitive impairment; (3) No history of neurological or psychiatric illness; (4) No severe visual and auditory impairment interfering the cognitive assessment.

This study was approved by the Institutional Ethical Committee of Xuanwu Hospital. Informed consent was obtained from each subject either directly or indirectly from his or her guardian.

Neuropsychological Background Tests

Both case and control subjects completed the Chinese version of MMSE (20), CDR (21), and FAB (22) to assess the global cognition and frontal lobe function. The bvFTD and AD patients also received a battery of neuropsychological tests to assess the: (1) Executive function: the Trail Making Test A (TMT-A) and B (TMT-B) (23), Digit Span Test forward (DST-F) and backward (DST-B) (24); (2) Episodic memory: modified World Health Organization-University of California Los Angeles Auditory Verbal Learning Test Immediate Recall (AVLT-I) and Delayed Recall (AVLT-D) (25); (3) Language: Boston Naming Test (BNT) (26), and Animal Fluency Test (AFT) (27); (4) Neuropsychiatric status: Frontal Behavioral Inventory (FBI) (28) and Geriatric Depression Scale (GDS) (29).

Mini Social Cognition and Emotional Assessment (Mini-SEA)

All participants undertook the Chinese version of the mini-SEA to evaluate the social cognition and emotion performance. The English version of the mini-SEA (16), provided and authorized by professor Bertoux., was translated and adapted into Chinese (mandarin) by two neurologists (W.F., Z.A.) and one psychologist (Z.L.). Then, the Chinese draft was translated back into English by another independent psychologist working in an English-speaking country, and the back translation version was sent to Professor Bertoux. After full communication with Professor Bertoux, the Chinese version of the mini-SEA was established.

The mini-SEA is composed of two parts, a shortened version of the Faux-Pas test (FPT) and a reduced facial emotion recognition test (FERT). In the FPT, 10 social scenes (+1 example scene) are presented, including 5 faux-pas stories (scored from 0 to 30) and 5 control stories without a faux pas (scored from 0 to 10). Patients were required to detect and explain the social inconveniences in the stories. Some adaptations were required according the Chinese cultural: (1) The English name in all the stories was changed to Chinese name; (2) The "blonde hair" in story 4 was changed to "black hair"; (3) The dialogue at the end of story 5 was modified to the following sentence: Her boyfriend said "Never mind. Don't give up. You can get an opportunity next time." She said: "Alright, I will keep studying hard."; (4) "The book he wanted about hiking in the Grand Canyon" in story 6 was changed to "a travel book about Beijing"; (5) "apple pie" in story 7 was changed to "apple cake". In the FERT, 35 faces with 7 different facial emotions (happiness, surprise, neutral, sadness, fear, disgust, and anger) are presented (scored from 0 to 35). Patients were required to recognize emotions of faces. The 35 face stimuli in the Chinese version of mini-SEA have been selected from the Chinese Facial Affective Picture System (CFAPS) to match the 35 white faces from the original FERT in terms of intensity, age, emotions and gender (30). The total FPT and FERT scores then converted to the composite subscores (scored from 0 to 15 respectively). The overall mini-SEA composite score was obtained by adding the two composite subscores (scored from 0 to 30).

The Chinese version of mini-SEA showed good validity and reliability. Validity was assessed by content validity index (CVI). Three experts were invited to evaluate each story and each picture. The S-CVI/UA (scale-level CVI based on the universal agreement method) were 0.83, and the S-CVI/Ave (scale-level CVI based on the average method) was 0.94. Reliability was assessed by intraclass correlation coefficient (ICC). Inter-rater reliability was performed on 30 subjects, including 10 bvFTD patients, 10 AD patients, and 10 controls. All subjects assessed by two raters simultaneously. For half of them, rater 1 asked questions, while rater 2 looked on and scored their performance. For the other half, rater 1 and rater 2 switched their roles. All subjects were re-evaluated by the rater who asked questions in the first rating after 4 weeks. The test-retest reliability was ICC 0.85 for the mini-SEA, and the inter-rater reliability was 0.92. The Cronbach's alpha coefficient was 0.71 for the FPT, 0.87 for the FERT, and 0.74 for the mini-SEA. The Chinese version of mini-SEA in this study were completed by two experienced well-trained raters, who received a standardized training. Mini-SEA was scored blind to the other instruments.

Statistical Analysis

The normality of the demographic and neuropsychological data for the three groups was tested using Shapiro-Wilk method. To facilitate comparison with previous research results, all data were expressed as mean $\pm$ standard deviation (SD). Parametric data (age at visit and education) were analyzed across three groups by analysis of variance (ANOVA), followed by *post-hoc* tests for pairwise comparison (Scheffe method). The student's *t*-test was used to compare means of age at onset and duration between bvFTD and AD group. If the normal distribution is not satisfied (neuropsychological data), Kruskal-Wallis test was used to compare the three groups, followed by Mann-Whitney test for two-by-two comparisons. Bonferroni correction was used for multiple comparisons. The adjusted *P*-value was obtained by multiplying the original *P*-value by the number of comparisons ($N = 3$). Differences in gender among three groups were assessed

by the Pearson Chi-square test. These statistical analyses were performed using SPSS 20.0 software.

Receiver operating characteristics (ROC) curves were used to evaluate the discriminatory power of each test by calculating the area under the curve (AUC). Cut-off points were determined using Youden's index to select the point giving best sensitivity and specificity. The difference between two ROC curves were calculated by a nonparametric method (Delong's method). The ROC analyses were performed by MedCalc Statistical Software 18.2.1. All statistical tests were two sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Demographic Data and General Neuropsychological Tests

The demographic data of bvFTD, AD (MCI+MD) and control groups are shown in **Table 1**. There were no statistically significant differences in gender and education level among the three groups (all $P > 0.05$). The mean age of the bvFTD patients was not statistically different from that of the controls ($P > 0.05$), but the AD patients was significantly older than the bvFTD patients (69.15 *vs.* 62.95, $P = 0.027$) or controls (69.15 *vs.* 62.93, $P = 0.015$). The mean age of onset of the bvFTD group was significantly earlier than that of the AD (MCI+MD) group (59.77 *vs.* 66.92, $P = 0.007$).

Compared with the control group, MMSE and FAB scores were significantly worse in the bvFTD ($Z = -5.208$, adjusted $P < 0.001$ and $Z = -4.919$, adjusted $P < 0.001$, respectively) and AD patients ($Z = -6.132$, adjusted $P < 0.001$ and $Z = -3.426$, adjusted $P = 0.002$, respectively). However, MMSE scores did not significantly differ between the bvFTD and AD (MCI+MD) group ($P > 0.05$). The bvFTD patients had lower FAB scores than AD, suggesting more frontal dysfunction in bvFTD, though the differences did not reach statistical significance ($Z = -1.597$, adjusted $P = 0.331$). Not surprisingly, worse performance of the FBI tests was seen in bvFTD than AD ($Z = 5.813$, $P < 0.001$). No significant difference was observed in GDS scores between the bvFTD and AD (MCI+MD) groups ($P > 0.05$).

Performance of the Mini-SEA and Other Cognitive Tests

The mini-SEA scores were significantly different among bvFTD, AD (MCI+MD) and control groups ($H = 46.940$, $P < 0.001$), as well as scores of FTP ($H = 37.749$, $P < 0.001$) and FERT ($H = 36.406$, $P < 0.001$) (**Table 2**). Pairwise comparison showed that the mini-SEA scores of the bvFTD group were significantly worse than those of the control group ($Z = -6.850$, adjusted $P < 0.001$) and AD (MCI+MD) group ($Z = -3.737$, adjusted $P = 0.001$). The bvFTD patients also showed significantly more damage on both the FPT and FERT than controls ($Z = -6.092$, adjusted $P < 0.001$ and $Z = -6.030$, adjusted $P < 0.001$, respectively) and AD patients ($Z = -4.070$, adjusted $P < 0.001$ and $Z = -3.185$, adjusted $P = 0.004$, respectively). The FERT and mini-SEA tests was slightly impaired in the AD (MCI+MD) group compared with the control group ($Z = -2.873$, adjusted $P = 0.012$ and $Z = -3.136$, adjusted $P = 0.005$, respectively) and there was no difference on the FPT scores between the two groups ($P > 0.05$). After controlling the age, the statistical results on the mini-SEA were not affected.

There were no statistically significant differences in TMT-A, TMT-B, DST-F, DST-B, AVLT-I, and AFT scores between the bvFTD and AD (MCI+MD) groups (all $P > 0.05$). However, bvFTD patients significantly performed better than AD patients on the AVLT-D test ($Z = 3.597$, $P < 0.001$), and worse on the BNT test ($Z = -2.000$, $P = 0.046$). After controlling the age, the statistical results on the neuropsychological tests were not affected.

ROC Analysis for the Mini-SEA, FPT and FERT

ROC analysis (**Table 3**) showed that the mini-SEA had a high discriminatory power for differentiating the bvFTD patients from the controls, with an AUC value of 0.989 (95% $CI = 0.905–1.000$, $P < 0.001$), as well as FPT (AUC = 0.954, 95% $CI = 0.857–0.993$, $P < 0.001$) and FERT (AUC = 0.953, 95% $CI = 0.856–0.992$, $P < 0.001$). The sensitivity and specificity were 95.5% and 93.3% respectively with a cut-off value at 21.4 for the mini-SEA.

The AUC value of the mini-SEA for differentiating bvFTD from AD was 0.899 (95% $CI = 0.777–0.967$, $P < 0.001$), with 81.8% sensitivity and 96.2% specificity at a cut-off value of 18.9. However, the sensitivity and specificity of FERT (cutoff = 9.4) were <80%, obviously lower than that of FPT and mini-SEA, though the difference in AUC values was not statistically significant ($P > 0.05$).

Comparisons of ROC Curves for the Mini-SEA, AVLT-D, BNT and FAB

The AUC values of the AVLT-D, BNT and FAB for differentiating bvFTD from AD was 0.793 (95% $CI = 0.650–0.897$, $P < 0.001$), 0.685 (95% $CI = 0.524–0.819$, $P = 0.046$) and 0.691 (95% $CI = 0.540–0.818$, $P = 0.026$) (**Figure 1**). The AUC value of the mini-SEA was higher than that of the AVLT-D, BNT and FAB, though the differences were statistically significant only between the mini-SEA and BNT (0.899 *vs.* 0.685, $Z = 2.249$, $P = 0.025$). Then, we combined the mini-SEA and AVLT-D, two tests with AUC values higher than 0.7. The discriminatory power of the new indicator was significantly better than that of AVLT-D (0.946 *vs.* 0.793, $Z = 2.333$, $P = 0.020$), BNT (0.946 *vs.* 0.685, $Z = 2.690$, $P = 0.007$) and FAB (0.946 *vs.* 0.691, $Z = 2.283$, $P = 0.022$).

DISCUSSION

This study established strict inclusion and exclusion criteria in accordance with internationally recognized diagnostic criteria (4), and all the enrolled patients were in the mild stage of the disease. The bvFTD group was mostly based on the clinical diagnosis, and only 3 patients diagnosed with evidence of gene mutations. The AD group included MCI individuals with evidence of amyloid deposition and mild dementia patients. We modified the English version of the mini-SEA by adopting Chinese facial affective pictures in FERT and making some

TABLE 1 | Demographic characteristics and general neuropsychological test data for the bvFTD, AD and control groups.

Characteristics	bvFTD $N = 22$	AD (MCI+MD) $N = 26$	Controls $N = 30$	bvFTD vs. AD	bvFTD vs. Controls	AD vs. Controls
Gender (F/M)	11/11	14/12	17/13	NS	NS	NS
Age at visit, years	62.95 ± 8.59	69.15 ± 8.50	62.93 ± 6.36	$P = 0.027$	NS	$P = 0.015$
Age at onset, years	59.77 ± 9.00	66.92 ± 8.65	–	$P = 0.007$	–	–
Duration, months	29.05 ± 17.91	26.85 ± 16.08	–	NS	–	–
Education, years	12.18 ± 3.29	10.92 ± 3.16	11.80 ± 3.04	NS	NS	NS
MMSE	24.23 ± 3.25	23.54 ± 2.01	29.07 ± 1.08	NS	$P < 0.001$	$P < 0.001$
FAB	14.14 ± 3.09	16.08 ± 1.41	17.50 ± 0.63	NS	$P < 0.001$	$P = 0.002$
CDR-SOB	4.00 ± 1.26	3.00 ± 1.20	0	NS	$P < 0.001$	$P < 0.001$
FBI	26.95 ± 8.87	7.23 ± 4.11	–	$P < 0.001$	–	–
GDS	6.18 ± 6.29	6.04 ± 4.45	–	NS	–	–

Data expressed as number of subjects or mean ± SD.
MMSE, mini-mental state examination; FAB, frontal assessment battery; CDR, clinical dementia rating; SOB, sum of boxes; FBI, frontal behavioral inventory; GDS, geriatric depression scale; NS, not significant.

TABLE 2 | Performance of Mini-SEA and cognitive tests on executive function, episodic memory and language for the bvFTD, AD and control groups.

Tests	bvFTD $N = 22$	AD (MCI+MD) $N = 26$	Controls $N = 30$	bvFTD vs. AD	bvFTD vs. Controls	AD vs. Controls
mini-SEA	15.79 ± 3.77	21.86 ± 2.14	24.27 ± 1.90	$P = 0.001$	$P < 0.001$	$P = 0.005$
FPT	7.56 ± 2.45	11.34 ± 1.64	12.40 ±1.48	$P < 0.001$	$P < 0.001$	NS
FERT	8.24 ± 2.02	10.52 ± 1.56	11.87 ± 1.04	$P = 0.004$	$P < 0.001$	$P = 0.012$
Executive function						
TMT-A	77.88 ± 31.22	68.15 ± 28.50	–	NS	–	–
TMT-B	190.00 ± 84.36	168.65 ± 86.31	–	NS	–	–
DST-F	7.41 ± 1.33	7.58 ± 1.10	–	NS	–	–
DST-B	4.24 ± 1.48	3.96 ± 0.96	–	NS	–	–
Episodic memory						
AVLT-I	18.10 ± 6.20	16.42 ± 4.49	–	NS	–	–
AVLT-D	4.43 ± 3.23	1.19 ± 2.14	–	$P < 0.001$	–	–
Language						
BNT	18.63 ± 5.20	21.62 ± 4.16	–	$P = 0.046$	–	–
AFT	11.20 ± 4.23	12.19 ± 3.52	–	NS	–	–

Data expressed as mean ± SD.
mini-SEA, the abbreviated version of the social cognition and emotional assessment; FPT, faux-pas test; FERT, facial emotion recognition test; TMT, trail making test; DST, digit span test; AVLT, auditory verbal learning test; BNT, boston naming test; AFT, animal fluency test; NS, not significant.

adaptations to the stories of FPT according the characteristics of Chinese cultural. The results showed that both bvFTD and AD had social cognitive impairment, involving ToM and emotional recognition, in the early stages. However, the damages in bvFTD were more prominent and extensive than those in AD. Consistent with Bertoux's results (17), the Chinese version of mini-SEA had good sensitivity and specificity for differentiating mild bvFTD from controls or mild AD. To our knowledge, this is the first study to evaluate the social cognitive impairment of bvFTD in Chinese population. Our results suggests that clinical features of bvFTD are comparable across different ethnicities, and the Chinese version of mini-SEA can be used as an effective tool for early diagnosis of bvFTD in Chinese population. It is worth mentioning that the average scores of the Chinese version of mini-SEA in the control group were slightly lower than those of Bertoux's studies (24.3 *vs.* 25.8), and this trend was also seen in the both bvFTD and AD group. The discrepancy may come from cultural differences among ethnic groups. Cross-cultural research is necessary to understand social cognitive in health and neurodegenerative cognitive disorders.

The mini-SEA is composed of FPT and FERT, two tests on ToM and emotion recognition respectively. Then, the two subscores of mini-SEA were further analyzed in this study. The patients with bvFTD showed more significant impairment on the FPT than AD patients and controls, while no difference was seen between AD patients and controls. This was similar to what previous studies had reported. In 2002, Gregory et al. (31) first assessed ToM performance in patients with bvFTD and AD. They found that bvFTD patients had significant deficits on the FPT and other ToM tests, which closely correlated to the ventromedial frontal atrophy, while AD patients only showed deficits on memory-based questions. Subsequent research confirmed this

TABLE 3 | ROC analysis for Mini-SEA, FPT and FERT to discriminate the bvFTD group from the controls or AD group.

Tests	Groups	AUC	95% *CI*	SE	*P* value	Cutoff	Sensitivity	Specificity
mini-SEA	bvFTD *vs.* Controls	0.986	0.905–1.000	0.011	< 0.001	≤ 21.4	0.955	0.933
	bvFTD *vs.* AD	0.899	0.777–0.967	0.049	< 0.001	≤ 18.9	0.818	0.962
FPT	bvFTD *vs.* Controls	0.954	0.857–0.993	0.028	< 0.001	≤ 9.8	0.864	0.900
	bvFTD *vs.* AD	0.891	0.767–0.962	0.052	< 0.001	≤ 9.8	0.864	0.846
FERT	bvFTD *vs.* Controls	0.953	0.856–0.992	0.028	< 0.001	≤ 10.3	0.909	0.900
	bvFTD *vs.* AD	0.809	0.670–0.908	0.062	< 0.001	≤ 9.4	0.773	0.692

ROC, receiver operating characteristics; mini-SEA, the abbreviated version of the social cognition and emotional assessment; FPT, faux-pas test; FERT, facial emotion recognition test; AUC, area under the curve; CI, confidence interval; SE, standard error.

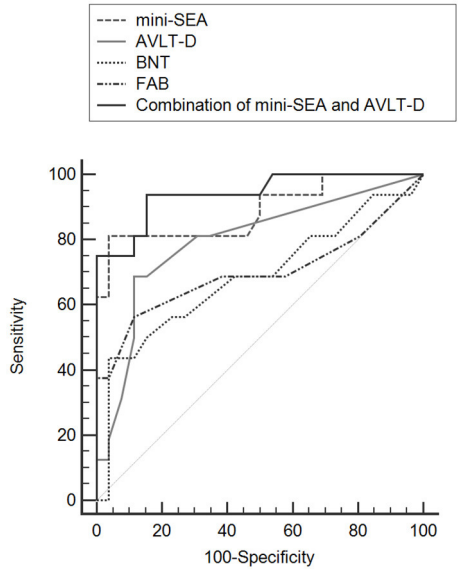

FIGURE 1 | Comparisons of ROC curves for mini-SEA, AVLT-D, BNT, and FAB for discriminating bvFTD from AD.

idea (32, 33) and showed the FPT was not only a sensitive diagnostic indicator in early bvFTD than other cognitive measures (34, 35), but also a specific method that distinguished bvFTD from AD (36, 37). However, a recent longitudinal multicenter study did not find the difference on the baseline FPT scores between bvFTD and other neurodegenerative diseases (38). More longitudinal studies with larger samples are needed to confirm the diagnostic specificity of the FPT. Another important aspect of social cognition is emotion recognition, and FERT is one of the most commonly used tasks to assess it. Previous studies have shown that FERT scores in bvFTD patients were significantly lower than those in both control subjects (39–43) and AD patients (44–46). Meanwhile, facial emotion recognition was slightly impaired in early AD compare with controls, and progressively declined over the course of the disease (45). Meta-analysis showed that facial emotion recognition was significantly impaired in bvFTD, especially in negative emotions including anger, disgust, fear and sadness (47). The results of our study were consistent with the findings of previous studies, supporting the idea that bvFTD affected the facial emotion recognition in the early stage, and more profound impairment in emotion recognition were presented in bvFTD than that in AD patients.

In this study, we also assessed executive function, episodic memory and language in patients with bvFTD and AD. It has been proposed in the current diagnostic criteria that executive dysfunction was the core cognitive deficit in bvFTD. However, it is sometimes difficult to accurately distinguish AD from bvFTD using traditional executive function tests even in the early stages of the disease (6, 7). Consistent with previous studies, our results showed that there were no statistically significant differences in the TMT, DST and FAB tests between the two diseases. We found that the AVLT-D score in bvFTD was significantly higher than that in AD. It is generally accepted that episodic memory is relatively preserved in the early stages of bvFTD, though recent studies have shown that memory impairment also occur in the early stage of bvFTD (8, 48). Longitudinal results showed that measures on executive function and memory strongly overlapped for bvFTD and AD, thus did not accurately discriminate the two diseases (49). Previous studies showed that scores of the mini-SEA was correlated with gray matter volume within the medial prefrontal cortex (mPFC) (50, 51). Meanwhile, the atrophy in the mPFC is the most characteristic neuroanatomical changes in the early stage of bvFTD, and helps distinguish bvFTD from other types of neurodegenerative dementia. Therefore, social cognitive impairment is considered as crucial cognitive signatures of bvFTD, just as episodic memory deficits are the core symptom of AD. Supporting this view, the mini-SEA showed a better discriminatory power comparing with AVLT-D, BNT or FAB in this study, and a combination of tests for mini-SEA and AVLT-D might have the greatest ability to discriminate bvFTD from AD.

There are several limitations in this study. First, the sample size was small, which would influence the research outcomes. However, our samples can provide a power of 90% to detect the difference in the mini-SEA scores between bvFTD and AD, and a power of 99% to detect the diagnostic values of ROC curves for the mini-SEA at a significance level of 0.05. Second, AD patients were not matched on age with the bvFTD patients and controls. Since the age at onset of bvFTD is earlier than that of AD, this problem is likely to occur if patients are continuously collected over a relatively short period of time. Fortunately, the statistical results on neuropsychological data were not affected after controlling the age. Previous studies have shown that ToM and facial emotion recognition got worse with age (52, 53).

Therefore, the older age in the AD patients might decreased the mini-SEA scores, but did not substantially affect the conclusion of our study. Third, not all patient's diagnoses were supported by evidence of CSF/PET biomarkers. Only the patients diagnosed as MCI due to AD or bvFTD with memory loss underwent the lumber puncture for AD biomarker measures or amyloid-PET to confirm or exclude AD. Last, social cognitive is impaired in many other diseases, such as schizophrenia, depression, Parkinson's disease, Huntington's disease, etc. Therefore, future studies with larger sample size, more types of disease and multiple biomarkers are needed to confirm the diagnostic value of the Chinese version of mini-SEA in bvFTD.

CONCLUSION

This study revealed that compared with AD, bvFTD had more significant social cognitive impairment and relatively retained memory in the early stage of the disease. The Chinese version of mini-SEA is a good clinical tool for the early diagnosis of bvFTD, and has a high sensitivity and specificity to discriminate bvFTD from AD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethical Committee of Xuanwu Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FW and JJ: study concept and design. FW, AZ, CW, XZ, and XM: acquisition of clinical data. LZ and HJ: acquisition of neuropsychological data. FW and YL: analysis of data and statistical analysis. FW, AZ, CW, XZ, DG, and JJ: drafted or revised the manuscript. FW: acquired financial support. All authors contributed to the article and approved the submitted version.

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A Color-Picture Version of Boston Naming Test Outperformed the Black-and-White Version in Discriminating Amnestic Mild Cognitive Impairment and Mild Alzheimer's Disease

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Despite the ubiquity of the Boston naming test (BNT) in clinical practice and research, concerns have been expressed about its poor quality pictures, insufficient psychometric properties, and cultural bias in non-English language backgrounds. We modified the black-and-white BNT with a set of color pictures since color effects have been suggested to improve naming accuracy in the visual naming test. This study aimed to examine and compare the reliability and validity of the color-picture version of BNT (CP-BNT) and the black-and-white version of BNT (BW-BNT) to differentiate amnestic mild cognitive impairment (aMCI) or mild Alzheimer's disease (AD) from the cognitive normals. This study included two subgroups, and each subgroup had 101 normal controls, 51 aMCI, and 52 mild AD. One subgroup undertook BW-BNT and the other conducted CP-BNT. The reliability, convergent and discriminant validity, and the diagnostic accuracy of two versions of BNT were evaluated. The CP-BNT showed a greater area under the curve (AUC) than the BW-BNT for aMCI (80.3 vs.s 69.4%) and mild AD (93.5 vs. 77.6%). The CP-BNT also demonstrated better convergent validity with CDR global scores and better reliability (Cronbach's coefficient 0.66 for the CP-BNT vs. 0.55 for the BW-BNT). At the optimal cutoff value of spontaneous naming, the CP-BNT demonstrated improved sensitivity and specificity for differentiating mild AD from NC with a higher positive predictive value, negative predictive value, and lower false-positive rate. Compared with BW-BNT, CP-BNT is a more reliable and valid test to assess cognitive and naming impairment.

Keywords: Boston naming test, Alzheimer's disease, mild cognitive impairment, naming deficit, language impairment

INTRODUCTION

Naming difficulty is the most common symptom of language dysfunction seen in neurological diseases such as Alzheimer's disease, frontotemporal dementia, stroke, encephalitis, temporal lobe epilepsy, brain trauma, etc. (1). Language neuroscience research suggests that visual confrontation naming relies on specific and distributed brain networks that operate on sequential cognitive processes, spanning from visual recognition, semantic activation, lexical retrieval, and articulation of word form. Given the complexity of visual naming processes, damage in several cortical and/or subcortical regions would result in naming impairment (1). As a result, naming impairment could be used as a marker of clinical feature and severity of disease, as well as the predictor of stability of neurological disease (2).

The Boston naming test (BNT) is the most commonly used neuropsychological instrument for visual confrontation naming (3, 4). In the prodromal stage of AD, BNT was regarded as a valuable tool to characterize disease severity and track clinical progression. Longitudinal studies supported that naming impairment provided evidence for the clinical stability and diagnostic reliability of amnestic mild cognitive impairment (2, 5). Individuals with amnestic MCI who had naming deficits had more than twice the risk of converting to dementia than those who had single-domain amnestic MCI (2).

Despite the widespread popularity of the BNT in clinical practice and research, there has been a longstanding controversy about BNT. These arguments are that the BNT has inadequate sensitivity to detect subtle naming impairment, out-of-date line drawings, problematic psychometric properties, and cultural bias, especially used in Non-English language backgrounds (6, 7). For example, the 30-item BNT had an acceptable sensitivity in a sample of patients with mild AD, but poor sensitivity when applied in a clinical setting of MCI (AUC = 0.87; 61% sensitivity and 89% specificity) (8). Studies suggested some items of BNT had low difficulty and weak ability to discriminate between persons with low vs. high naming ability (9). This means that the BNT needs some modifications to improve its psychological properties.

The Boston naming test has also been criticized for the poor quality of its black-and-white line drawings (6). In Chinese clinical application, both examiners and examinees often complain that even normal subjects may have difficulties recognizing some original items of BNT. Since the BW-BNT was developed in 1983 and stayed largely unchanged in its format since then, many line drawings in the original version differed obviously from the target objects in the current time (3, 4, 10). There may be possible cohort effects as well as cultural bias that make the black-and-white line drawings liable to be misperceived in non-English language backgrounds. For example, the drawing of a "pretzel" is often misperceived as a snake (6). The "igloo" is usually mistaken for a mud oven due to its resemblance in some parts of South America (11). From an ecological view, the diagnostic validity of studies using black-and-white line drawings has been questioned (12). Consequently, the number of naming tests using color stimuli, which provided a more realistic representation of objects, has been progressively increasing (13–15). A recent meta-analysis of 35 studies suggested that color information had a color effect on object recognition to improve naming accuracy and speed correct response times (16).

The 30-item Chinese version of BNT has been adapted several times since the 1990s. Given that Chinese is a logographic language, phonemic cueing is not applicable to this population. The adaptation in 2004 developed a new word choice cuing paradigm to replace the phonemic cue (17). The reliability and validity of the 30-item Chinese version of BNT have been validated (18, 19), but the application of BNT is still limited by its poor psychometric properties and cultural bias. For the need for greater sensitivity and cultural appropriateness in the neuropsychological assessment, we modified the Chinese version of the 30-item BNT with a set of color pictures to replace the original black-and-white line drawings. The study aims to determine and compare the reliability, convergent and discriminant validity, and diagnostic accuracy of CP-BNT and BW-BNT in a Chinese sample with cognitive normals, mild cognitive impairment patients due to Alzheimer's disease, and mild Alzheimer's disease.

METHOD

Participants and Diagnosis

All participants (aged 55–85 years) included normal cognitive control, patients with amnestic mild cognitive impairment (aMCI), and mild Alzheimer's disease (mild AD). Patients were consecutively recruited from the memory and language clinic, Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing from January 2015 to December 2018. The normal controls were volunteers from the community and the spouses or caregivers of patients. They were community-dwelling, cognitive, and neurologically healthy individuals.

A guideline for referral to neuropsychological assessment was the MMSE score $\geq$20 at the initial visit to the clinic, but patients with lower MMSE scores may also be referred if deemed relevant (e.g., in the case of low education). All participants had an extensive diagnostic assessment including a clinical interview, neurological and physical examination, a comprehensive neuropsychological assessment battery published previously (20), routine laboratory examinations (comprising vitamin B12 and folate dosage, serology for syphilis, and thyroid hormones), and brain structural scans (MRI and/or CT). Subjects were excluded from the study if any of the following conditions applied: (1) evidence of stroke or Fazekas score $\geq$2, as determined by neuroradiological or clinical examination; (2) history of severe head injury; (3) current psychiatric diagnosis; (4) any medical condition that leads to severe cognitive deterioration, including thyroid dysfunction, diabetes, renal, respiratory, cardiac, and hepatic disease; (5) present or past abuse or daily use of alcohol or drugs. After completion of the diagnostic workup, the multidisciplinary staff established a consensus diagnostic classification.

An aMCI patient was diagnosed according to the clinical core criteria of MCI due to AD proposed by the National Institute on Aging-Alzheimer's Association workgroups (NIA-AA) (21). The criteria included: (1) memory impairment with

an insidious onset and gradual progression; (2) an objective memory impairment was defined by impaired performance on the Chinese version of WHO/UCLA-AVLT delayed recall below the cut-off value (≤9) (20); (3) score higher than the cutoff points for dementia in MMSE, >26 for middle school and above; (4) clinical dementia rating (CDR) of 0.5, with a score of at least 0.5 on the memory box; (5) ability preserved to perform daily activities and social functions; (6) neuroimaging features consistent with incipient AD (i.e., hippocampus and entorhinal cortex atrophy) and no other lesions; and (7) no other medical or neuropsychiatric conditions that could account for the cognitive impairment. AD dementia was diagnosed according to the clinical core criteria of AD dementia proposed by NIA-AA, and only participants with mild AD dementia with a global CDR score of 1 were included (22).

These criteria resulted in 408 participants, including 202 cognitively normal controls, 102 participants with aMCI, and 104 with mild AD. All participants were randomly divided into two subgroups by cognitive level. Each subgroup had 101 NCs, 51 patients with aMCI, and 52 patients with mild AD. One subgroup undertook the black-and-white line drawing version of BNT, and the other subgroup performed the new color-picture version of BNT. Written informed consent was obtained from all participants and/or their families and approved by the ethical committee of Xuanwu Hospital.

The Development of the Color-Picture Version of BNT (CP-BNT)

The present study adopted the same items from the Chinese version of BNT published in 2004 (17, 19). Thirty color pictures were used to replace the black-and-white line drawings. Twenty-nine pictures were photographs of real objects. Only the picture for the item "sea horse" was a color drawing obtained from the internet. **Supplementary Figure 1** demonstrated the pictures of the item "broom" in BW-BNT and CP-BNT. The change in picture format did not change the target response word. As a result, the CP-BNT did not change the word frequency and familiarity, the number and length of syllables, and difficulty of articulation for the 30 items. All the color pictures were placed on a plain white or plain colored background, and the mean dimension of the images was 265 x 223 pixels. The CP-BNT was displayed on 19-inch LCD color monitors with a screen resolution of 128 x 800 pixels and a 64-bit color mode.

The Administration of the CP-BNT and the BW-BNT

All participants were administered all 30 items of the CP- BNT or the BW-BNT, starting from item 1, with no setting of a basal or discontinuation rule. Each participant was asked to name the picture with a single word as precisely as possible once it appeared on the computer screen (CP-BNT) or the paper card (BW-BNT). If the participant named the item correctly, the examiner proceeded to the next item. If the participant gave a wrong response or gave no response within 20 s, then a semantic cue was given. If the participant could not name the object correctly with the semantic cue, the three-choice recognition task was given, and the choice of the examinee was recorded.

The measures of naming performance included the total number of correct items on spontaneous naming, semantic cuing, and word recognition. Scores of spontaneous naming (SN) were computed as the number of items correctly named spontaneously, ranging from 0 to 30. The score of semantic cueing (SC) is the number of correct responses after giving the semantic cue, as well as the score of word recognition (WR), ranging from 0 to 30. Two additional descriptive scores, the percentage of correct responses on semantic cuing or word recognition (SC and %WR), were calculated for all the participants by dividing the number of correct responses during semantic cuing or recognition by the number of errors preceding the cues, ranging from 0 to 100%. The total score of BNT is the sum of the scores of SN, SC, and WR, ranging from 0 to 30.

Statistical Analyses

The Chi-square and Mann–Whitney U tests were used to examine the differences in demographic characteristics, clinical cognitive function, and performance of two versions of BNT between the two subgroups by the same cognitive level (i.e., normal control, aMCI, and mild AD, respectively). Within each subgroup, the Kruskal–Wallis test was used to examine the differences in demographic characteristics, cognitive function, and BNT performance among the three cognitive levels. The convergent validity of two versions of BNT was assessed by both univariable and multivariable regression analysis for the association between SN score and demographic and cognitive factors. For each subgroup, the area under the curve (AUC) with SN raw score was identified with receiver operating characteristics (ROC) curve analysis to differentiate aMCI or mild AD from NC. The SN optimal cut-off score was calculated based on Youden's J index (sensitivity + specificity −1). We also evaluated sensitivity and specificity, false-positive rate (FPR), positive predictive value (PPV), negative predictive value (NPV), and Likelihood ratio for a positive test result (LR+) at the optimal SN cut-off value for aMCI and mild AD in the two subgroups.

To adjust for the effect of demographic variables (age, sex, and education) on the diagnostic accuracy, the T-score of SN was calculated based on the normal control demographically in the two subgroups, respectively. A ROC analysis with an adjusted T-SN score was conducted for aMCI and mild AD in the two subgroups.

The reliabilities of the two versions were examined using Cronbach's alpha coefficients, respectively. We considered $p < 0.05$ (two-sided) as the statistical significance. Statistical analyses were performed using SPSS Statistics version 22.0 (IBM Corp., Armonk, NY) for Windows.

RESULTS

Demographic Characteristics and Clinical Cognitive Function

Table 1 shows the demographic characteristics and clinical cognitive function of the two subgroups (six diagnostic groups). There was no significant difference in the demographic

TABLE 1 | Demographic, cognitive characteristics, and performance of BNT in the two subgroups.

	Subgroup 1 with BW-BNT			Subgroup 2 with CP-BNT		
	NC ($n = 101$)	aMCI ($n = 51$)	Mild AD ($n = 52$)	NC ($n = 101$)	aMCI ($n = 51$)	Mild AD ($n = 52$)
Male (%)	48 (47.5%)	27 (52.9%)	31 (59.6%)	47 (46.5%)	21 (41.2%)	22 (42.3%)
Age (ys)	65.6 (5.9)	67.2 (6.7)	67.9 (7.2)[b*]	66.1 (6.0)	69.0 (5.6)	69.5 (7.1) [b**]
Education (ys)	12.7 (2.6)	12.3 (2.8)	12.2 (2.7)	13.0 (2.7)	11.3 (3.2)	11.8 (3.5) [b**]
MMSE	28.5 (1.1)[a**]	26.2 (1.4) [a**]	21.4 (2.2) [b**]	29.1 (1.2)	26.4 (2.1)	20.7 (2.4) [b**]
MoCA	26.0 (1.5) [a**]	20.2 (1.9) [a**]	15.9 (2.3) [b**]	26.6 (1.6)	21.8 (2.7)	16.2 (2.9) [b**]
CDR	0 (0)	0.5 (0)	1 (0)	0 (0)	0.5(0)	1 (0)
Spontaneous naming	25.3 (2.8) [a**]	23.1 (3.2) [a**]	22.0 (3.2) [b**]	27.6 (1.5)	24.6 (3.0)	23.0 (2.9) [b**]
Semantic cueing	1.0 (1.1) [a**]	1.3 (1.4)	1.1 (1.6)	0.4 (1.6)	0.6 (0.8)	0.8 (0.9) [b**]
%Semantic cue	22.0 (24.9) [a**]	15.9 (19.8)	15.4 (19.0)	13.3 (25.3)	12.9 (20.3)	12.4 (14.8)
Word recognition	3.1 (2.3) [a**]	4.4 (2.5)	5.1 (2.9) [b**]	1.9 (1.5)	4.3 (2.8)	5.1 (2.8) [b**]
%Word recognition	78.3 (32.0) [a*]	75.4 (25.7) [a*]	72.4 (25.5) [b*]	80.0 (37.6)	82.2 (27.9)	81.0 (22.5) [b*]
Total score of BNT	29.4 (0.9) [a**]	28.7 (1.3) [a**]	28.2 (1.7) [a**,b**]	29.9 (0.4)	29.5 (0.6)	28.9 (1.2) [b**]

Data are presented as mean (SDs) or number (proportion %).
BW-BNT, Black-and white version of Boston naming test; CP-BNT, Color-picture version of Boston naming test; NC, Normal control; AD, Alzheimer's disease; aMCI, amnestic mild cognitive impairment; MMSE, mini-mental status examination; MoCA, Montreal cognitive assessment; CDR, Clinical dementia rating.
[a] *Significantly different from cognitive matched groups.*
[b] *Significantly different within the BW-BNT or CP-BNT.*
** $p < 0.05$; ** $p < 0.01$.*

characteristics between the two subgroups based on cognitive levels. Compared with subgroup 1, NC and aMCI patients in subgroup 2 had significantly higher MMSE and MoCA scores. There was no statistically significant difference in MMSE and MoCA scores between patients with mild AD in the two subgroups.

When compared within subgroups, normal controls were significantly younger than patients with aMCI and mild AD ($p = 0.026$ for subgroup 1, $p = 0.002$ for subgroup 2). In subgroup 2, normal controls had significantly higher education than patients with aMCI and mild AD ($p = 0.006$).

Differences in Performance Between the BW-BNT and the CP-BNT

As shown in **Table 1**, compared with subgroup 1 with BW-BNT, normal controls in subgroup 2 with CP-BNT scored significantly higher in SN ($p< 0.01$), %WR ($p< 0.05$), and total BNT ($p < 0.01$) with a significant decrease in SC, %SC, and WR. At the MCI level, patients with CP-BNT maintained significantly higher scores in SN ($p < 0.01$), %WR ($p < 0.05$), and total BNT ($p < 0.01$). However, at the mild dementia level, patients with CP-BNT only scored higher significantly in total BNT ($p < 0.01$). The differences in SN, SC, %SC, WR, and %WR were not significant.

Results of Regression Analysis (Convergent Validity)

In subgroup 1 with BW-BNT, the univariable regression analyses showed that SN was significantly associated with gender, education, the MoCA, and CDR global score. Multiple stepwise regressions, however, revealed that only the MoCA ($\beta = 0.45, p < 0.001$) and gender ($\beta = -0.19, p = 0.002$) significantly predicted the SN score.

In the CP-BNT subgroup, the univariable regression analyses showed that SN was significantly associated with gender, education, the MMSE, MOCA, and CDR global score. However, using the multiple stepwise regressions, age ($\beta = -0.21, p < 0.01$), education ($\beta = 0.17, p < 0.01$), the MoCA ($\beta = 0.27, p = 0.018$), and CDR ($\beta = -0.33, p < 0.01$) significantly predicted the SN score. The results of univariable regression analyses are shown in the **Supplementary Tables 1**, **2**.

Results of Receiver Operating Characteristic Curve With SN Raw Score

Figure 1 shows the area under the curves for aMCI and AD for the two versions of BNT with SN raw score. For both aMCI and mild AD, CP-BNT demonstrates greater AUC than BW-BNT.

As shown in **Table 2**, for CP-BNT, the optimal SN cut-off score of 25/26 yielded a sensitivity of 60.8% and a specificity of 90.1% for aMCI vs. NC with an AUC of 80.3% (95%CI: 72.4–88.4%), and a sensitivity of 80.8% and a specificity of 90.1% for mild AD with an AUC of 93.5% (95%CI: 89.6–97.2%).

For the BW-BNT, the optimal SN cut-off score of 25/26 yielded a sensitivity of 74.5% and a specificity of 54.5% for differentiating aMCI from NC with an AUC of 69.4% (95%CI: 60.7–78.1%). At the optimal cutoff of 22/23, the SN raw score differentiated NC and mild AD with a sensitivity of 63.5%, specificity of 82.2%, and an AUC of 77.6% (95%CI: 69.9–85.3%). **Table 2** also shows that the CP-BNT outperformed the BW-BNT in PPV, NPV, and LR+, both for aMCI and mild AD. Also notably, the CP-BNT had much lower False positive rate (FPR) than the BW-BNT for aMCI (9.9 vs. 45.5%) and mild AD (9.9 vs. 17.8%).

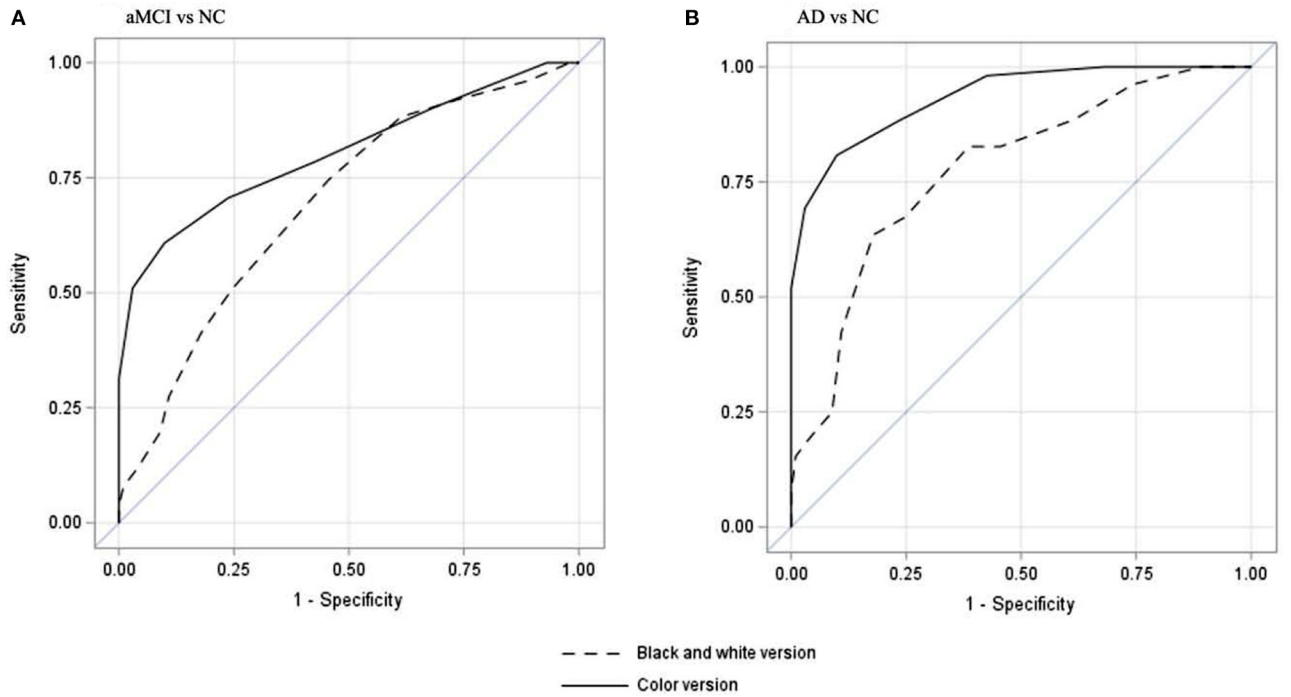

FIGURE 1 | Receiver operating characteristic curves of spontaneous naming score to differentiate aMCI from NC **(A)** and to differentiate mild AD from NC **(B)**. **(A)** showed that CP-BNT had a better AUC (80.3%, 95%CI: 72.4–88.4%) than that of BW-BNT (AUC = 69.4%, 95%CI: 60.7–78.1%) to differentiate aMCI from NC. **(B)** showed that CP-BNT had a better AUC (93.5%, 95%CI: 89.6–97.2%) than that of BW-BNT (AUC =77.6% (95%CI: 69.9–85.3%) to differentiate AD from NC.

TABLE 2 | The diagnostic and differentiating ability of CP-BNT and BW-BNT.

BNT version	Cut-off value	Sensitivity	Specificity	AUC (95%CI)	FPR	PPV	NPV	LR+
CP-BNT								
aMCI vs. NC	25/26	60.8%	90.1%	80.3% (72.4; 88.4)	9.9%	75.6%	82.0%	6.1
AD vs. NC	25/26	80.8%	90.1%	93.5% (89.6; 97.2)	9.9%	80.8%	90.1%	8.2
BW-BNT								
aMCI vs. NC	25/26	74.5%	54.5%	69.4% (60.7; 78.1)	45.5%	45.2%	80.9%	1.6
AD vs. NC	22/23	63.5%	82.2%	77.6% (69.9; 85.3)	17.8%	64.7%	81.4%	3.6

CP-BNT, the color picture version of Boston naming test; BW-BNT, the black-and-white version of Boston naming test; aMCI, amnestic mild cognitive impairment; NC, normal control; AD, Alzheimer's disease; AUC, Area under the ROC curve; CI, Confidence interval of the AUC; TPR, true positive rate; FPR, false positive rate; PPV, positive predictive value; NPV, negative predictive value; LR+, Likelihood ratio for a positive test result.

The ROC Analysis With the Adjusted SN Score

The AUC with adjusted SN scores for aMCI was 85.5% (95%CI: 79.4–91.7%) with the CP-BNT and 71.2% (95%CI: 62.3–80.0%) with the BP-BNT. The AUC of the adjusted SN scores for mild AD was 91.4% (95%CI: 86.5–96.3%) with the CP-BNT and 81.4% (95%CI: 74.1–88.6%) with the BP-BNT. The results also supported that CP-BNT improved diagnostic accuracy for aMCI and mild AD in comparison with BW-BNT. To facilitate the clinical application of CP-BNT, we adopted the SN raw score instead of the adjusted SN score in the present study for further discussion.

Reliabilities of the Two Versions of BNT

The CP- BNT had an acceptable Cronbach's coefficient ($\alpha = 0.66$). Compared with the CP-BNT, the BW-BNT had a poor Cronbach's coefficient ($\alpha = 0.55$).

DISCUSSION

In the present study, we modified the black-and-white version of the 30-item BNT with a set of color pictures. We examined and compared the psychometric properties of the CP-BNT with the BW-BNT in a Chinese sample of cognitive normals and patients with aMCI and mild AD. The results supported that the CP-BNT

outperformed the BW-BNT for detecting aMCI and mild AD with higher reliability, validity, and diagnostic accuracy in the Chinese language background.

Color is represented in a structural representation system and forms an essential attribute of the conceptual knowledge of prototypical objects. According to previous studies, the presence of color and photographic detail assists the processing of visual confrontation naming in terms of improving naming accuracy and speeding the response time (15, 23). It is generally thought that color effects arise at both the visual processing level and the semantic level, where color not only facilitates the visual recognition of objects but also provides additional cues to activate its semantic knowledge of prototypical objects (16, 24). In the present study, CP-BNT also demonstrated the color effect with higher SN scores and total scores than that of BW-BNT, as well as better diagnostic accuracy for detecting aMCI and AD. Besides the color effect, the CP-BNT adopted target pictures that were common in the everyday life of the Chinese.

These adaptations improved the cultural appropriateness of BNT in Chinese language background, which might further reduce the possibility of misperception. Consequently, in the present study, the CP-BNT showed a significantly lower false-positive rate than the BW-BNT in aMCI (9.9 vs. 45.5%) and mild AD (9.9 vs. 17.8%).

However, the color effect gradually diminished as the cognitive impairment deteriorated from NC to MCI and then to mild dementia. The same results were reported by Adlington et al. that naming for elderly controls improved linearly with color information, while patients with AD showed no benefit from the addition of color (25). One possible explanation is the early and common visual association cortex atrophy reported in patients with AD, especially atrophy of the angular gyrus and posterior middle temporal gyrus, which are supposed to interact with the default mode network and connect the visual recognition process with the semantic representations in the anterior temporal lobe (26, 27). Furthermore, Harnish et al. reported that visual discrimination abilities of objects strongly predicted performance on both picture naming and semantic association abilities in AD, but lacked the same predictive value for the controls (27). It may be, therefore, the case that the CP-BNT showed better discriminant ability to differentiate aMCI and mild AD from NC than the BW-BNT in the present study. The results also added indirect evidence that the visual deficit plays a role in the naming impairment in AD.

Results of stepwise regression models suggested that the CP-BNT had better convergent validity with the CDR global score than the BW-BNT. Patients with mild AD tended to perform worse in CP-BNT than aMCI patients, which suggests CP-BNT might be used as a better marker for disease deterioration than BW-BNT. Moreover, CP-BNT was significantly associated with age and education, while BW-BNT was only related to gender. Since BNT is a cultural and language relevance test, a majority of studies have found that age and education level are particularly related to the performance of BNT (28). However, in the Chinese sample, the effect of age and education on BNT was still controversial (18, 19). The discrepancies could be attributed to varying research methods and participant characteristics (e.g., age range, educational levels) (28). However, in the present study, the two subgroups had matched demographic characteristics and the same language background. The improvement in psychometric properties of BNT and better convergent validity was likely due to the changes in the picture format, which improved the picture quality and cultural appropriateness.

Collectively, our study offers methodological strengths. Although a few studies have supported that visual naming tests with color stimuli have better ecological validity than tests with black-and-white material (13–15), this is the first study to modify BNT with color pictures. It is also the third adaptation of the Chinese version of BNT to make it more culturally appropriate in the context of the Chinese language (17). The results supported empirically that CP-BNT outperformed the BW-BNT by overcoming some shortcomings such as low quality of pictures, poor psychometric properties, cultural bias, and thus improved the diagnostic accuracy for detecting aMCI and mild AD.

It is important to note that the current study has several limitations. First, our sample size was limited and from a clinic-based sample. The results of normal controls cannot be truly representative of the general population. Second, as the current sample was likely to be younger and well-educated, samples with limited education should be cautious to use the SN cutoff value of 25/26, which may result in an overestimation of the naming deficit. Third, the CP-BNT can be believed to have better diagnostic accuracy for detecting aMCI, although the sensitivity (60.8%) is still not optimal. The results also revealed that some items showed a marked ceiling effect. The value of this test lies in its ability to detect the severe to moderate level of naming deficit rather than its standalone ability to identify specific patients with aMCI (29). For the patients with single-domain amnestic mild cognitive impairment, episodic memory appears to be affected before other cognitive domains, although those individuals who manifest impairments in one or more cognitive domains are more likely to convert to dementia (2, 30).

In summary, the current study provided empirical evidence supporting that CP-BNT outperformed BW-BNT in validity and reliability to detect patients with aMCI and AD. The study deepened our understanding of the psychometric properties of BNT and improved the manipulability in Chinese language background. We suggest that the CP-BNT could be an ecological alternative to the original BNT, especially, but not exclusively in the Chinese language background.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of Xuanwu Hospital, CMU. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DL and Y-YY: study concept and design. DL, Y-YY, LL, and FW: acquisition of clinical data. DL and LL: acquisition of neuropsychological data. NH: analysis of data and statistical analysis. DL, NH, LL, MZ, and FW: drafted or revised the manuscript. MZ: performed MR/CT brain scan and provided a neuroimaging diagnosis. L-MF and S-SR: contacted patients, family caregivers, and normal controls. DL: acquired financial support. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.884460/full#supplementary-material

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BRIEF RESEARCH REPORT
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Culture Effects on the Chinese Version Boston Naming Test Performance and the Normative Data in the Native Chinese-Speaking Elders in Mainland China

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Background: The Chinese version of Boston Naming Test (BNT-C) is administered in China widely. However, the neuropsychological parameter of BNT-C in native Chinese-speaking elders in mainland China has not been explored systematically. The aim of this study was to explore cultural influences on BNT-C performance and establish norms among native Chinese-speaking elders in Beijing.

Methods: A total of 161 native, Chinese-speaking, cognitively normal elders aged ≥55 years were enrolled from various communities in Beijing. The BNT-C was conducted on all the participants. The internal consistency, participants' familiarity, and naming accuracy were analyzed and compared with data from Chinese areas outside the mainland and from American published previously. The influencing factors and stratified norms for BNT-C were established.

Results: The BNT-C showed good internal consistency ($\alpha = 0.738$). Strong correlation between naming accuracy and object familiarity was found ($r = 0.962$, $P < 0.001$). Participants' familiarity and correct naming rate for many items were notably different between the Chinese-speaking elders and English-speaking elders in America. The difference in some items' correct naming rate also existed between Beijing, Taiwan, and Hongkong. Higher education was associated with higher scores, whereas age and gender had no effect on BNT-C performance. The recommended norms of total naming scores for elders with education ≤9 and >9 years were 16 and 23, respectively.

Conclusion: The participants' familiarity with BNT items differed between different cultures, which further affected the naming accuracy and total scores. The education stratified norms established here are helpful for the better application of BNT-C in mainland China.

Keywords: Boston Naming Test, confrontation naming, cross-cultural, lexical familiarity, normative data, Chinese population, elders

INTRODUCTION

The Boston Naming Test (BNT) was compiled by Goodglass and Kaplan in 1983, which is composed of 60-line drawings of objects and animals, ranging from very familiar objects (trees and pencils) to unfamiliar objects (Sphinx and scaffolding) (1). By far, it is the most widely used confrontational naming test in the world and provides valuable diagnostic information for patients with aphasia and other cognitive-linguistic impairments from stroke (2), head injury (3), and neurodegenerative diseases such as frontotemporal dementia (4).

The BNT was originally designed for English-speaking people in North America. Individuals' familiarity with BNT items differs among culture, populations, and countries, which likely affect the naming performance and total scores (5). Therefore, it is important to explore the item familiarities and establish BNT norms according to local cultural and linguistic populations when used outside North America (5). So far, BNT has been adapted to many languages, including Danish (6), Spanish (7), Chinese (8), Dutch (9), Korean (10), French Canadian (11), Greek (12), Italian (13), Malay (14), and Swedish (15). Among the various versions of the BNT in different languages, some of them adopted the original English items (6, 15, 16) and others made adjustments or replacements to some items to adapt to the local cultural background (12, 17, 18). Even in other English-speaking countries outside America, such as Australia (19), BNT also had to be adapted to local populations.

The Chinese version of BNT (BNT-C) was developed by Hongkong scholars by selecting 30 items from the original English version without item adjustment and replacement (20). The authors proved that BNT-C successfully distinguished naming impairment in Cantonese-speaking patients with a head injury from a normal control group. Since then, BNT-C has been widely used across China. Due to cultural differences, people's familiarity with BNT items varies between the Chinese and Caucasian populations. For example, the igloo and harp are relatively unfamiliar to the Chinese, while the abacus, which is considered the most difficult for Americans, is well-known to many Chinese. Consequently, the influential factors and norm data of the BNT-C may differ markedly from those in the west. However, there has been no study exploring the familiarity of the BNT-C. The per-item correct naming rate and striated norm of BNT-C in China mainland have not been evaluated and reported either. In this study, we administered BNT-C to 161 Chinese-speaking community elders in Beijing. The participants' familiarity and naming performance for each BNT-C item were determined and compared with those from American (21) as well as other Chinese areas (Taiwan and Hongkong) published previously (5, 20). The correlations between naming accuracy and familiarity were further explored. The effects of demographic variables (gender, age, and education) on naming performance were examined, and stratified norms were established considering significant influential factors.

METHODS

This study was conducted from January 2018 to November 2019 at five communities in Beijing, China. The study protocol was approved by the Ethics Review Board of the Xuanwu Hospital, Beijing Capital Medical University. Written informed consent was obtained from all the participants.

Subjects

The participants were recruited from community volunteers in Beijing. The eligible samples for inclusion were (1) 55–85 years old, (2) native Chinese speakers, and (3) the Mini-Mental State Examination (MMSE) $\geq$24 (22). Neurologists interviewed all the subjects. Any individuals with a history of psychiatric or central nervous system diseases, hearing loss, learning disability, and any other condition that was likely to have an influence on performance in the BNT were excluded.

Chinese Version of BNT

The BNT-C used in this study consists of 30 items (**Table 2**) selected from the original 60 items without item adaption (20). The order of presentation followed the original sequence, and the design of the stimulus cards was identical to that of the original pictures (20).

Procedures and Scoring

The BNT-C was administered to all the participants by trained raters as described by Cheung RW who, together with his colleagues, developed the BNT-C test (20). All 30 cards bearing the line-drawing objects or animals were presented to the elders in a fixed order. Participants were instructed to name each object depicted on the cards. If the participant named one item correctly, one point was awarded and it was recorded among "scores of spontaneous naming (SN)." The examiner then proceeded to the next item. If the participant gave a wrong response or gave no response within 20 s, the participant's response was recorded in detail and a standard semantic cue was provided (e.g., "it is a plant" for "tree"). A semantic cue was designed for each item as in the original version of BNT. If the participant gave the correct answer, one point was awarded and it was recorded among "scores after semantic cue (SC)" (20). In the original BNT, if the participants failed the semantic cue, then a phonemic cueing was supplied. Given that Chinese is a logographic language and the names of most objects consist of one sound, BNT-C adopted a multiple-choice recognition task including the target response, name of an object similar to the target in function (e.g., "cow" for "camel"), and name of an object similar to the target in appearance (e.g., "mountain" for "camel"). The participant's response was recorded but no credit was given for choosing naming. The total score of BNT-C ranged from 0 to 30 ("SN" plus "SC"), the higher indicating the better naming ability.

Familiarity Rating

The participants were instructed to rate each item of BNT-C for familiarity based on how usual or unusual the objects

were in their experience, using a 5-point scale ranging from 1 (very unfamiliar) to 5 (very familiar). Familiarity was defined as "the degree to which you come in contact with or think about the object."

Statistical Analysis

Statistical analyses were performed with SPSS version 24.0 (SPSS Inc., Chicago, Ill., USA). Demographic and neuropsychological data were presented as mean ± SD or number and percentage. The mean data between two groups were analyzed using an independent-sample *t*-test or χ^2 test (chi-square). The internal consistency of BNT-C was assessed by Cronbach's alpha. Pearson's correlation coefficient was used to determine the correlation between naming accuracy and familiarity. A multiple linear regression analysis was used to explore the influence of age, gender, and education on BNT-C performance. For all tests, $P < 0.05$ was considered statistically significant.

RESULT

In total, 161 cognitively normal elders, 74 men (46.0%) and 87 women (54.0%), were recruited for the current study. The average age was 71 years (range: 55–85 years). The average period of formal education was 11.4 years (range: 1–19 years). The average MMSE score was 28.03 (range: 25–31). Demographic data and MMSE scores stratified by education were presented in **Table 1**.

Internal Consistency of the BNT-C

The 30 items composing the BNT-C showed a high-internal reliability coefficient ($\alpha = 0.738$). Every item in the BNT-C was positively correlated with the total score and contributed positively to Cronbach's α for the total score.

Per Item Familiarity and Correct Naming Rate of BNT-C

Participants' familiarities for each BNT-C item were recorded and compared with those from a study that rated the 60 BNT pictures on familiarity in 30 elder native English speakers in America (21). However, the study adopted a scale ranging from one (not at all familiar) to seven (very familiar) instead of one to five. To facilitate comparison, we multiplied their results by five-sevenths. The average familiarity of Chinese in our study was 4.33, but it was 4.94 in the American residents. Our elders rated not familiar (<4) in seven items (igloo, harp, pyramid, seahorse, dart, cactus, and trellis) and rated familiar or very familiar in the remaining 23 items. American elders rated all 30 items as familiar or very familiar. Further analysis into items with a familiarity difference >0.5 showed that Americans were much more familiar with igloos than Chinese (4.98 vs. 1.95). They were also more familiar with harp (5 vs. 3.07), pyramid (4.98 vs. 3.35), cactus (5 vs. 3.79), dart (4.86 vs. 3.71), seahorse (4.81 vs. 3.67), and trellis (4.93 vs. 3.83). The familiarities with rhinoceros, harmonica, tongs, protractor, and tripod were also 0.5 points higher in the Americans than in the Chinese residents. Abacus was the only item that the Chinese residents were more familiar with than the Americans (4.80 vs. 4.55) (**Table 2**).

Per item correct naming rates for the 30 BNT-C items were recorded and compared with data from American normal elders (23) and other Chinese normal elders outside the mainland [Taiwan (5) and Hong Kong (20)]. The correct naming rate difference between populations >20% was considered significant (5) (**Table 2**). Compared with the American residents, Beijing elders performed better in naming for compass, abacus, and protractor but performed worse in naming igloo, harp, dart, trellis, seahorse, cactus, and pyramid. The naming accuracy gap of igloo, harp, dart, protractor, compass, and abacus between the Chinese and the Americans was close to or >40%. Only 3.9% of the Beijing elders named igloo correctly compared with 98% of the American residents. In contrast, American residents got 55% and 46.7% correct naming rates for abacus and compass, respectively, lower than those of the Beijing residents (97.1 and 93.2%, respectively). Compared with the Hong Kong elders, the Beijing residents performed better in naming mushroom and protractor but worse in seahorse and dart. The Beijing residents named better in protractor and worse in seahorse, igloo, harp, and trellis than the Taiwan elders. Compared with the American elders, the Beijing, Taiwan, and Hong Kong elders were consistently good in naming compass and abacus but consistently worse in igloo, dart, and harp. All populations performed well in naming tree, pencil, scissors, flowers, racquet, hanger, camel, saw, snail, funnel, escalator, wheelchair, with a correct rate of >90%.

Pearson correlation analysis was used to explore the correlation between correct naming and familiarity. The Pearson correlation coefficient was 0.962 ($P < 0.001$), which indicated that naming accuracy was highly correlated with familiarity.

Influential Factors and Stratified Norms for BNT-C

Multiple linear regression analysis was used to explore the effect of age, gender, and education on BNT-C performance (**Table 3**). No significant correlations between age or gender and BNT-C scores were found. The analysis identified that education strongly correlated with BNT-C performance. Therefore, the subjects were subgrouped into the following four educational categories: (1) ≤6 years ($n = 32$); (2) 7–9 years ($n = 40$); (3) 10–12 years ($n = 42$), and (4) ≥13 years ($n = 47$). According to the *post hoc* analysis, there were no significant differences between ≤6 years and 7–9 years of education and between 10–12 years and ≥13 years of education in scores for spontaneous naming (≤6 vs. 7–9 years: 20.29 ± 3.95 vs. 22.66 ± 3.31; 10–12 vs. ≥13 years: 25.00 ± 2.28 vs. 25.30 ± 2.37) and total scores after semantic cueing (≤6 vs. 7–9 years: 22.29 ± 3.58 vs. 23.94 ± 3.49; 10–12 vs. ≥13 years: 26.38 ± 1.83 vs. 26.65 ± 1.85). Accordingly, we subgrouped the participants into two education level groups (≤9 and >9 years) eventually.

The naming accuracy was significantly higher in the high education group (>9 years) than in the lower education group (≤9 years) in seahorse (77.00 vs. 42.90%, $P < 0.001$), dart (68.90 vs. 47.60%, $P = 0.031$), rhinoceros (95.10 vs. 78.60%, $P = 0.010$), harp (54.10 vs. 26.20%, $P = 0.005$), pyramid (86.90 vs. 47.60%, $P < 0.001$), compass (100.00 vs. 83.30%, $P = 0.004$), tripod (90.20 vs. 52.40%, $P < 0.001$), tongs (91.80 vs. 71.40%,

TABLE 1 | Demographic and neuropsychological data.

	Total	Education (years)		t	*P*-value
		≤9	>9		
Number	161	72	89	/	
Male: female ratio	74:87	31:41	43:46	0.443	0.506
Age, years	71.00 ± 6.29	70.70 ± 6.69	71.19 ± 6.06	−0.414	0.680
Education, years	11.41 ± 3.54	7.74 ± 1.46	13.76 ± 2.22	15.245	<0.001
MMSE	28.03 ± 1.65	27.45 ± 1.90	28.43 ± 1.33	−3.058	0.003
BNT-C scores (SN)	23.83 ± 3.37	21.79 ± 3.75	25.14 ± 2.33	−5.488	<0.001
BNT-C scores (TS)	25.26 ± 3.10	23.36 ± 3.65	26.47 ± 1.88	−5.405	<0.001
BNT-C cut-off score (SN)		14	20		
BNT-C cut-off score (TS)		16	23		

MMSE, Mini-Mental State Examination; BNT-C, the Chinese version of the Boston Naming Test; SN, scores of spontaneous naming; TS, total scores ("scores of spontaneous naming" plus "scores after semantic cue").

$P = 0.006$), and protractor (85.20 vs. 57.10%, $P = 0.001$) (**Table 4**). Consequently, the high education group achieved better spontaneous naming scores (25.14 ± 2.33 vs. 21.79 ± 3.75, $P < 0.001$) and total scores (26.47 ± 1.88 vs. 23.36 ± 3.65, $P < 0.001$) than the lower education group (**Table 1**). We chose "mean-2*SD" as the recommended cut-off value. For the individuals with formal education >9 years, the appropriate cutoff was 20 for spontaneous naming scores and 23 for total scores. For the individuals with formal education ≤9 years, the cutoff scores were 14 and 16, respectively.

DISCUSSION

BNT was originally developed for the English-speaking populations in America. Because of its practicability and simplicity, many scholars have translated and adapted BNT for people with different languages and cultural backgrounds. BNT-C was composed by selecting 30 items from the original English version and is widely used in China. However, the psychometrics of the BNT-C has been rarely reported on and evaluated in the native Chinese-speaking elderly in the mainland. This study explored the internal consistency, per item familiarity, and correct naming rate of the BNT-C for the first time and generated striated norms for the Chinese-speaking elders in mainland China. We found good internal consistency in BNT-C, indicating that the items of this test reliably measure the same construct. Compared with the Americans, the Chinese elders were significantly less familiar with many items in the BNT-C and achieved low naming accuracy and total scores. We identified a positive correlation between education and BNT-C performance and established norms according to different education levels.

Object Familiarity and Naming Accuracy of BNT-C in Different Cultural Backgrounds

BNT consists of 60 items for participants to name, including common things such as beds and trees, as well as uncommon objects such as pyramids and sphynx. People vary in their familiarities with each item due to different cultural backgrounds, life experiences, and education levels. However, familiarity data have been rarely evaluated and reported previously both in China and other countries. The current study found that the Chinese elders were not familiar with igloo, harp, pyramid, seahorse, dart, cactus, and trellis, which are not common in daily life or ordinary readings in China. Ferraro et al. rated the BNT pictures on familiarity in 30 elder native English speakers in America (21). As expected, the Chinese residents were less familiar with many items than the American residents. Most Chinese elders were very unfamiliar with igloo, which was very familiar to the Americans. In contrast, the Chinese residents were more familiar with abacus which was less familiar to the Americans. Many Chinese elders used the abacus for calculation in their early lives. The discrepancies in familiarities for BNT items truly reflected the cultural difference between China and America. Besides BNT-C, there were many adapted versions outside North America. Most modified versions replaced part test items according to local cultures (12, 17, 18). It seems appropriate to consider the possibility of replacing items that are not suitable for the Chinese individuals with more culturally representative items in the adapted Chinese BNT version. The development of new test items was outside the scope of this study, but future research should certainly address this consideration.

A previous study indicated that picture familiarity facilitates the processing of BNT word representations (24). It is expected that people likely name objects correctly when they are familiar with them. Consistently, we found a strong positive correlation between naming accuracy and item familiarity in the BNT-C performance. This correlation further explained the difference in per item correct naming rate between the Chinese and American residents. The American residents performed better in naming igloo, cactus, harp, and pyramid which they were more familiar with than the Chinese. In contrast, they perfomed significantly worse in naming abacus which they were least familiar with. The results further demonstrated how familiarity influenced naming accuracy.

BNT-C was developed by the Hong Kong scholars and was used in Taiwan and the mainland widely. Compared with people from Hong Kong or Taiwan, Beijing elders performed better

TABLE 2 | Per-item familiarity and correct naming rate of BNT-C in our study and other studies.

Item No.	Original item No.	Item	Familiarity		Correct (%)			
			BJ	USA (21)	BJ	HK (20)	TW (5)	USA (23)
Number			161	30	161	77	264	60
Age range			55–85	56–86	55–85	23–79	60–92	40–78
Education, years			11.4	15.0	11.4	9.7	12.5	13.9
SN			N/A	N/A	23.8 (3.4)	24.9 (3.0)	24.7 (3.9)	N/A
TS			N/A	N/A	25.2 (3.1)	26.7 (2.8)	N/A	54.5
1	2	Tree	4.84	5.00	100.0	100.0	100.0	100.0
2	3	Pencil	4.90	5.00	100.0	100.0	100.0	100.0
3	6	Scissors	4.92	5.00	100.0	100.0	100.0	98.3
4	8	Flowers	4.79	5.00	100.0	100.0	100.0	98.3
5	9	Saw	4.66	5.00	98.1	100.0	98.1	100.0
6	12	Broom	4.90	5.00	89.3	94.8	98.1	100.0
7	14	Mushroom	4.84	5.00	100.0	67.5	97.7	93.3
8	15	Hanger	4.91	5.00	99.0	100.0	99.2	100.0
9	16	Wheelchair	4.75	5.00	91.4	92.2	96.2	100.0
10	17	Camel	4.62	4.88	99.0	98.7	99.2	98.3
11	21	Racquet	4.76	5.00	100.0	97.4	98.1	100.0
12	22	Snail	4.60	4.95	98.1	90.9	96.2	100.0
13	24	Seahorse	3.67	4.81	63.1	90.9	82.6	91.7
14	25	Dart	3.71	4.86	61.4	83.1	73.9	98.3
15	30	Harmonica	4.24	5.00	80.6	83.1	84.5	86.7
16	31	Rhinoceros	4.13	4.95	88.4	81.8	93.6	83.0
17	33	Igloo	1.95	4.98	3.9	20.8	60.6	98.3
18	36	Cactus	3.79	5.00	71.8	85.7	86.4	100.0
19	37	Escalator	4.72	5.00	94.2	97.4	97.3	100.0
20	38	Harp	3.07	5.00	43.1	54.5	68.9	100.0
21	42	Stethoscope	4.67	5.00	95.2	83.1	87.9	96.7
22	43	Pyramid	3.35	4.98	70.9	83.1	79.5	95.0
23	46	Funnel	4.68	5.00	96.1	98.7	92.0	95.0
24	47	Accordion	4.57	4.95	96.1	77.9	84.8	91.7
25	50	Compass	4.63	4.91	93.2	90.9	92.0	46.7
26	52	Tripod	4.23	4.84	74.8	63.6	80.3	81.7
27	54	Tongs	4.20	4.95	83.5	81.8	96.6	81.7
28	57	Trellis	3.83	4.93	57.3	41.6	84.5	88.3
29	59	Protractor	4.16	4.81	73.8	33.8	44.7	35.0
30	60	Abacus	4.80	4.55	97.1	98.7	99.6	55.0

BNT-C, the Chinese version of the Boston Naming Test; "Original Item No." refers to the original BNT (1). Values for education and BNT are means. N/A, Not available; SN, scores of spontaneous naming; TS, total scores ("scores of spontaneous naming" plus "scores after semantic cue"); BJ, Beijing; HK, Hongkong; TW, Taiwan.

TABLE 3 | Results of multiple linear regression analysis of gender, age, and education on BNT-C score.

	Unstandardize B	Coefficients std. error	Standardized coefficients beta	t	*P*-value
(Constant)	24.466	3.949		6.195	<0.001
Gender	−1.043	0.587	−0.180	−1.778	0.079
Age	−0.002	0.055	−0.004	−0.041	0.967
Education	1.854	0.632	0.310	2.936	0.004

BNT-C, the Chinese version of the Boston Naming Test.

TABLE 4 | Percentage of correct BNT-C between two different education levels.

Item	Correct (%)		χ^2	*P*-value
	≤9 years	>9 years		
Tree	100.00	100.00	–	–
Pencil	100.00	100.00	–	–
Scissors	100.00	100.00	–	–
Flowers	100.00	100.00	–	–
Saw	95.20	100.00	2.96	0.164
Broom	88.10	90.20	0.00	0.992
Mushroom	100.00	100.00	–	–
Hanger	97.60	100.00	1.47	0.408
Wheelchair	95.20	88.50	0.69	0.406
Camel	100.00	98.40	0.70	1.000
Racquet	100.00	100.00	–	–
Snail	97.60	98.40	0.07	1.000
Seahorse	42.90	77.00	12.49	<0.001
Dart	47.60	68.90	4.68	0.031
Harmonica	76.20	83.60	0.87	0.35
Rhinoceros	78.60	95.10	6.59	0.010
Igloo	4.80	3.30	0.00	1.000
Cactus	64.30	77.00	2.00	0.157
Escalator	90.50	96.70	0.81	0.367
Harp	26.20	54.10	7.92	0.005
Stethoscope	92.90	96.70	0.19	0.667
Pyramid	47.60	86.90	18.58	<0.001
Funnel	92.90	98.40	0.81	0.367
Accordion	92.90	98.40	0.81	0.367
Compass	83.30	100.00	8.44	0.004
Tripod	52.40	90.20	18.82	<0.001
Tongs	71.40	91.80	7.49	0.006
Trellis	54.80	59.00	0.18	0.668
Protractor	57.10	85.20	10.16	0.001
Abacus	100.00	95.10	0.74	0.388

BNT-C, the Chinese version of the Boston Naming Test.

in naming protractor but worse in seahorse, dart, igloo, and harp. This was probably because the residents in Hong Kong and Taiwan were more influenced by western culture than the Chinese mainland residents. Compared with the Americans, the Chinese people including Beijing, Taiwan, and Hong Kong consistently performed better in naming compass and abacus but consistently worse in igloo, dart, and harp. This consistency reflected the long-term differences between the Chinese culture and the American culture.

Hobson et al. (25) showed a reliable creation of an estimated 60-item BNT score from administrations of the 30-item BNT by multiplying the obtained score by two. By this method, the total BNT scores from our study and those from Hong Kong were multiplied by the two to allow comparisons across different studies. The calculated total scores ("scores of spontaneous naming" plus "scores after semantic cuing") for elders in Beijing (estimated BNT score of 50) were lower than that in Hong Kong (20) (estimated BNT score of 53) and America (23) (estimated BNT score of 55). Combining the familiarity differences and the correlation between familiarity and naming accuracy, we could infer that cultural background influences participants' familiarities with BNT items, which affects the naming accuracy further and impact the total scores ultimately. However, it should be noted that the per-item familiarity and correct naming rate of BNT-C in this study and other studies in HK, TW, and the USA may have limited comparability given the varied age range and educational levels. Multicenter studies adopting the same inclusion criteria are required in future work for a more convincing comparison.

Effects of Demographic Factors on the BNT-C Performance

Demographic variables including age, gender, and education were repeatedly reported to impact the performance of cognition measures. Lower mean BNT scores with lower educational levels have been frequently found in the published works of literature (26–30). This study also demonstrated that education correlated significantly with BNT-C scores. Our findings were consistent with other research. Cheung et al. (20) performed the BNT-C in 77 normal adults in Hong Kong and found a positive association between education and naming scores ($r = 0.342$, $P < 0.01$). Chen et al. (5) applied the same BNT-C to 264 native Chinese people with normal cognition aged >60 years in Taiwan. They also found years of education were positively correlated with BNT-C score ($r = 0.376$, $P < 0.01$). Our research further showed that high-educated participants made fewer errors than low-educated subjects on dart, harp, pyramid, tripod, and protractor. The results were supported by the study exploring the influence of schooling on the performance of aphasia examination, which indicated that confrontation naming demands a greater degree of semantic knowledge, which is proved with increasing years of formal education (31). This may be because these objects are not common in daily life but are acquired gradually in study and reading.

The influence of gender and age on BNT performance remains controversial. Using the original 60-item BNT in normal elders in Middle Tennessee, Welch (30) found that age was significantly involved in confrontational naming ability. Moreover, there was also a gender bias that men scored significantly higher on 17 items than women. However, no gender effect was found for the Turkish version of the BNT (32) and the adapted BNT version for the Portuguese speakers (33). The Korean version of the BNT scores was slightly affected by age but rarely influenced by gender (10). For BNT-C, Cheung, who developed the BNT-C, found that gender and age have no effects on naming performance in Hongkong Chinese population (20). Chen et al. (5) did not identify the effect of either gender and age on BNT-C scores in the Taiwan residents. Consistent with previous research, we found no correlations between age or gender and BNT-C performance in this study. It likely indicated that gender and age affect the performance less on this 30-item BNT-C version.

This study has several limitations. First, our study mainly recruited the elders aged ≥55 years, so the data may not be generalized to the adults of all ages. Second, the major

subjects were educated and only few were illiterate. This might underestimate the education effects on BNT-C performance. However, with the popularization of compulsory education, there were fewer illiterates now, and there will hardly be illiterates in the future. The results of this study will be suitable for future use. Third, the number of participants in each age-education cell was relatively small. Further research should expand the sample size. Fourth, the participants were recruited from communities in the urban areas of Beijing. In the future, urban and rural residents should be selected nationwide to make the results more representative. Another one that should be considered was that the American, Hong Kong, and Taiwan studies used for comparison were conducted 10 years or more before. The results may deviate from the real differences between the current population. However, the Chinese people rated less familiar and score on many items than the American decades later, indicating that cultural differences between China and America and their impact on BNT-C persist till now.

CONCLUSIONS

To our knowledge, it was the first time to establish norm scores of BNT-C considering influencing factors in the elderly population of Chinese mainland. These scores presented here take into account subjects' education level and are therefore likely to help clinicians make diagnostic decisions more accurately. Further research should expand the sample size and explore the sensitivity and specificity of the BNT-C for the linguistic disorders in the native Chinese-speaking population. We also found a notable difference in participants' familiarity and naming accuracy for each BNT-C item between the Americans and native Chinese speakers, which was highly consistent with the cultural differences. The possibility of replacing items that are not suitable for the Chinese individuals with more culturally representative objects should be considered in future work.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Xuanwu Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AZ: conception, design, and revision of the article. YL, YQ, FW, and CW: data collection. YL: analysis and drafting of the article. RW, HJ, BX, and JY: data collection. JJ: revision of the article and approved the final version. All authors contributed to the article and approved the submitted version.

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Clinical Practice Guidelines for the Management of Behavioral and Psychological Symptoms of Dementia: A Systematic Review With AGREE II

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Background: High-quality clinical practice guidelines (CPGs) are important for the effective treatment of behavioral and psychological symptoms of dementia (BPSD). However, recommendations provided by different quality guidelines may lead to varied clinical practice outcomes.

Objective: To assess the quality of available CPGs for the management of BPSD and summarize the best recommendations for treating BPSD.

Methods: This was a systematic review of CPGs for the management of BPSD with data obtained from electronic databases and evaluated using the Appraisal of Guidelines for Research and Evaluation II instrument, consisting of six domains: "Scope and purpose", "Stakeholder involvement", "Rigor of development", "Clarity of presentation", "Applicability", and "Editorial independence". The criteria for high-quality guidelines were set as: the score of high-quality guidelines in the "Rigor of development" domain should be $\geq$60% and as well as a score of >60% in at least three other domains. High-quality guidelines were selected for recommendation extraction, and the final recommendations were formed in combination with the latest meta-analysis and randomized clinical-trial results.

Results: In term of median scores in each domain for the six included CPGs, "Scope and purpose" (87.5%) scored better than all others, whereas "Applicability" (46.5%) was the domain with the lowest score. Four CPGs (2015 APA, 2018 NICE, 2018 CANADA, 2020 EAN) met the criteria of high-quality guidelines and were used to extract recommendations. From these four CPGs, nine specific recommendations related to the management of BPSD were summarized, of which seven were related to pharmacological treatment and two to non-pharmacological treatment. These recommendations covered the applicability of antipsychotic

drugs, medication recommendations, withdrawal times, and several suitable non-pharmacological therapies.

Conclusion: The quality of CPGs for the management of BPSD requires improvement, especially for the "Applicability" domain. For psychotic-like symptoms in dementia, the use of antipsychotics should be based on the individual's risk-benefit ratio, and the use of atypical antipsychotics seems to be a better choice. Non-pharmacological treatments may be suitable for emotional symptoms and sleep disorders.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020209204.

Keywords: dementia, behavioral symptoms, practice guideline, antipsychotic agents, drug therapy

INTRODUCTION

Dementia has a higher prevalence among older individuals and currently affects 50 million patients worldwide, a number which is expected to increase to 152 million by 2050, according to the 2019 Alzheimer's disease International report (1). Meanwhile, more than 90% of patients experience behavioral and psychological symptoms of dementia (BPSD), including agitation, apathy, depression, repetitive questioning, psychosis, aggression, sleep problems, wandering, and various inappropriate behaviors (2). These symptoms are associated with decline in quality of life, poor functional status, and worsened cognition (3, 4). Therefore, the management of BPSD is of high medical and social priority. To assist physicians and patients in making decisions regarding the appropriate management of BPSD, different countries and regions have developed their own clinical practice guidelines (CPGs) through systematic reviews and assessments of the benefits and harms of different treatment options. However, the multiple available guidelines often lead to uncertainty for clinicians regarding which CPGs should be used to treat BPSD. Our work primarily aimed to synthesize high-quality evidence-based CPGs available for the management of BPSD. The secondary aim was to identify domains of therapeutic concern within the existing guidelines that can be further researched to improve future guideline development.

MATERIALS AND METHODS

The protocol of this systematic review was registered in the International Prospective Register of Systematic Reviews, with number CRD42020209204. This review was conducted in strict accordance with the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-analyses.

Data Sources and Searches

Two independent researchers conducted investigations using the following electronic bibliographic databases: PubMed, Cochrane Library, ClinicalKey, and UpToDate; as well as guideline specific sources: Canadian Medical Association Infobase, Guidelines International Network, National Institute for Health and Care Excellence (NICE), and Medlive. These were used to search for recent CPGs for the management of BPSD. We searched with terms such as "dementia", "behavioral and psychological symptoms", "behavioral symptoms", "psychiatric symptom", "guideline", and combinations. Azermai et al. (5) used the Appraisal of Guidelines for Research and Evaluation II (AGREE II) (6) tool to evaluate the quality of guidelines related to the management of BPSD from 2003 to 2012. We conducted the search for guidelines published from January 2012 to August 2020, and only in the English language. A flowchart of the search is shown in **Figure 1**.

Study Selection

First, the obtained results were imported into EndNote X9 to remove duplications. Then, studies were included after primary screening (read title and abstract) and secondary screening (read full text) of the above results. If any disagreement regarding whether to include an article occurred between the two independent reviewers, a third experienced review expert would be consulted.

We only included the most recent CPGs of international consensus, defined as documents developed by a nationally recognized committee, publicly funded institution, or medical society, providing recommendations for the management of BPSD, especially with respect to pharmacological treatment.

We excluded: (1) direct translations or interpretations of other guidelines; (2) documents where the full text was not available; (3) guidelines that solely provided recommendations on care; and (4) systematic reviews, letters to the editor, or frameworks of guideline development.

Quality Assessment

The quality of the screened guidelines was assessed using AGREE II, which is a widely used guideline evaluation tool (composed of 23 items within six domains) (6). AGREE II consists of six domains. The six domains are "Scope and purpose", "Stakeholder involvement", "Rigor of development", "Clarity of presentation", "Applicability", and "Editorial independence". Each domain is assessed based on several "items", with a total of 23 items. Each item is graded on a seven-point scale, with score of one indicating that the guideline does not comply with this item at all, and a score of seven indicating that the guideline fully complies with this item. The higher the score, the higher the compliance degree of the guideline in this item. Details of the items are

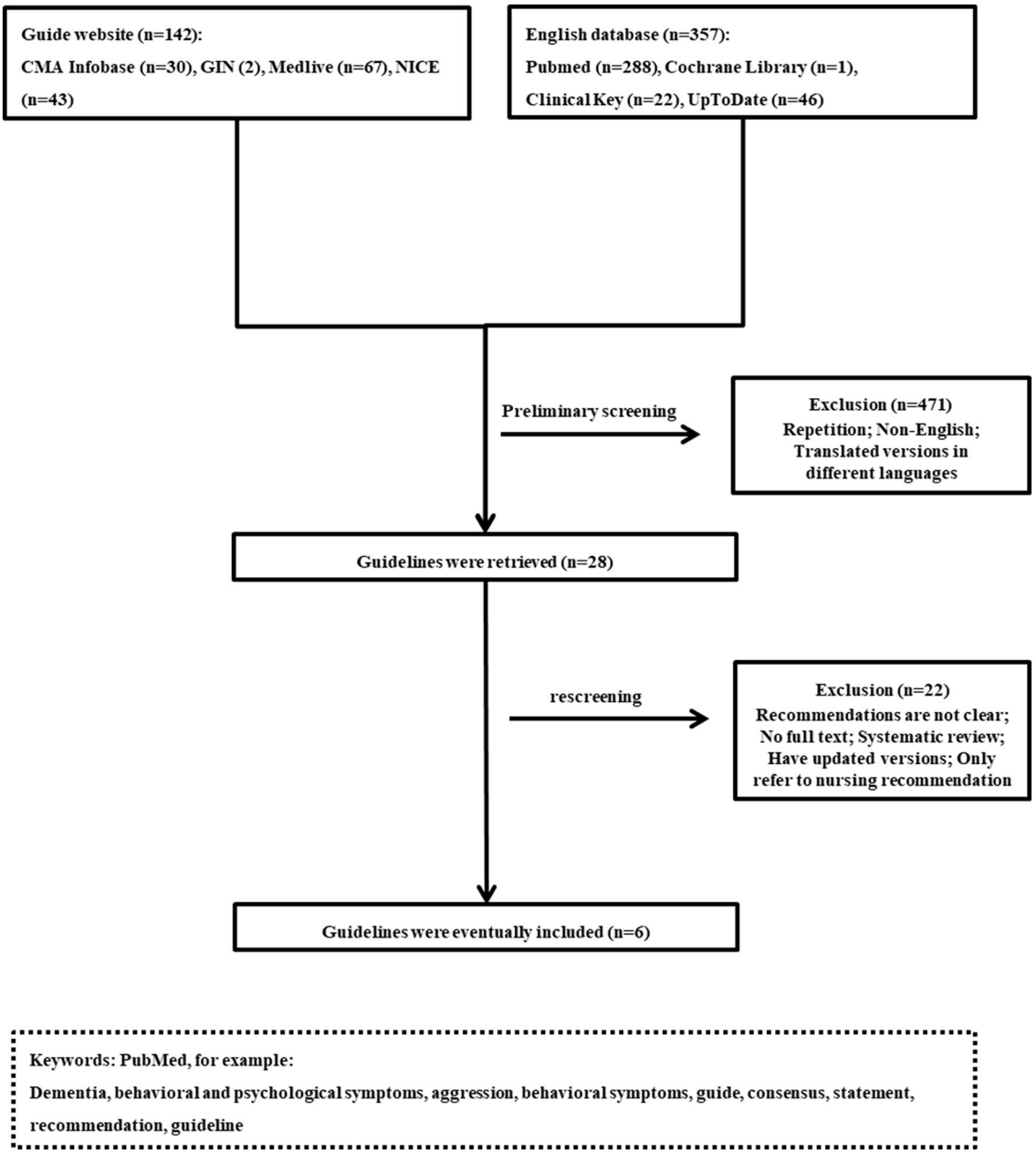

FIGURE 1 | The flowchart of the systematic search. (CMA Infobase), Canadian Medical Association Infobase; (GIN), Guidelines International Network; (NICE), National Institute for Health and Care Excellence.

available online (http://www.agreetrust.org/). The median score of the CPGs in each domain is an important indicator of the overall performance of the guidelines; hence, we calculated the median score of the guidelines in each domain. For screening high-quality guidelines, the "Rigor of development" domain was evaluated first. The score of the CPGs in the "Rigor of development" domain should be ≥60% (5, 7), and as well as a score of >60% in at least three other domains. Guidelines that did not meet these criteria were removed from further analyses.

Prior to the scoring process, the two reviewers conducted the training exercises available on http://www.agreetrust.org/. The scoring consistency between the two reviewers was computed using the intraclass correlation coefficient (ICC) with a two-way random effects model for each domain using SPSS software (version 26.0, IBM Corp., Armonk, NY). The ICC levels of consistency were categorized based on their respective scores in four classes: poor (<0.40), fair (0.40–0.59), good (0.60–0.74), or excellent (0.75–1.00) (8). If there were differences between the scores of the two reviewers, a third appraiser was consulted.

Data Extraction and Recommendation Generation

The two independent reviewers conducted information extraction on the six guidelines that passed the "Study Selection"

TABLE 1 | Clinical practice guideline characteristics and development methods for recommendations.

Guideline	Relevant content	Developers	Country/region	Target population	Type of evidence	Grading system
2015; APA	Use of antipsychotic medications when agitation or psychosis occurs in association with dementia	American Psychiatric Association	USA	Patients with dementia exhibiting agitation or psychosis	Randomized controlled trials, Systematic review, Expert opinion, Observational research, Patient values and preferences	YES
2018; DELPHI	Existing and emerging treatments for BPSD in Alzheimer's disease overall, as well as specifically for agitation and psychosis	Foreign expert group on psychiatry	International consensus panel	Patients with Alzheimer's disease exhibiting behavioral and psychological symptoms	Expert opinion, randomized controlled trials	NO
2018; IPS	Suggestions for the management of dementia	Indian Psychiatric Society	India	Patients with dementia	-	NO
2018; CANADA	When and how to safely taper and stop antipsychotics	Foreign expert group on psychiatry	Canada	Patients with behavioral and psychological symptoms of dementia and insomnia	Systematic reviews, expert consensus, randomized controlled trials	YES
2018; NICE	How dementia should be assessed and diagnosed	National Institute for Health and Clinical Excellence	United Kingdom	Patients with dementia	Systematic Reviews,Systematic review and Meta-analysis, Randomized controlled trials	YES
2020; EAN	Use of antipsychotics in dementia	European Academy of Neurology	Europe	Patients with dementia	Randomized controlled trials	YES

criteria. During this process, one of the reviewers independently extracted data from the six CPGs using a standardized form in MS Excel 2010 (Microsoft Corp., Redmond, WA). Target information included the title, year of publication, developers, country, target population, and Grade of Recommendations Assessment, Development and Evaluation (9) was used to evaluate the quality of each guideline (see **Table 1**). Then, the recommendations for the management of BPSD were extracted from the selected high-quality guidelines, along with the strength of recommendations and evidence supporting recommendations. The management of BPSD was the focus of each guideline. We extracted recommendations for the treatment of symptoms addressed by the guidelines, such as psychotic-like symptoms, emotional symptoms, and sleep disorders. Specific practice recommendations for BPSD were also classified into pharmacological and non-pharmacological interventions. Finally, the second reviewer inspected the extraction results. For the recommendations extracted from the four high-quality guidelines, we analyzed the consistency between the guidelines for the same recommendation, combined with the latest meta-analysis and randomized clinical trial results to form our final recommendations.

RESULTS

Systematic Search and Screening of CPGs

In total, 28 results were screened from 499 total results based on title and abstract information. After analyzing the full text, six CPGs met the inclusion criteria. The characteristics of these six CPGs are listed in **Table 1**. These CPGs were developed by the clinical and scientific bodies of the United States, European Union, Canada, United Kingdom, India, and an international consensus panel composed of individuals from different countries (including Europe, the United States, United Kingdom, Australia, and Canada); we termed them 2015 American Psychiatric Association (APA) guideline (10), 2018 DELPHI consensus (11), 2018 IPS guideline (12), 2018 CANADA guideline (13), 2018 NICE guideline (14), 2020 European Academy of Neurology (EAN) guideline (15).

Quality Appraisal of CPGs

Scope and Purpose

Domain 1 "Scope and purpose" assesses whether a guideline describes the overall purpose clearly, that is, whether a guideline describes its potential impact on patients and society when implemented to the specific clinical symptoms. Among the six included guidelines, the score of domain 1 was >60%, and the median score of domain 1 was the highest (87.5%) compared with those of the other five domains, suggesting that the guideline developers clearly elaborated on the overall purpose of the guideline (**Table 2**).

Stakeholder Involvement

Domain 2 "Stakeholder involvement" considers basic information regarding the guideline developers and the degree of participation of the audience in the guideline development process. Of the six included guidelines, four guidelines (2015 APA, 2018 NICE, 2018 CANADA, 2020 EAN) scored >60% in this domain, while two guidelines, 2018 DELPHI and 2018 IPS, scored low at 47% and 6%, respectively, because

TABLE 2 | Quality appraisal of dementia guidelines with the AGREE II instrument.

Included guidelines	Scope and purpose	Stakeholder involvement	Rigor of development	Clarity and presentation	Applicability	Editorial independence
2015 APA	94	75	79	92	63	96
2018 DELPHI	83	47	26	78	19	92
2018 IPS	75	6	19	72	33	0
2018 CANADA	92	86	81	92	71	83
2018 NICE	94	72	68	89	60	71
2020 EAN	78	58	60	72	29	75
Median	87.5	65	64	83.5	46.5	79

these two guidelines did not consider the audience's views through questionnaires or other methods during the guideline development process (item 5) and 2018 IPS did not provide basic information of the guideline developers (item 4).

Rigor of Development

In many studies, the score of domain 3 "Rigor of development" has been used as a criterion for assessing whether a guideline is of high quality. This domain requires the guideline to clarify the criteria for evidence retrieval and the process for developing recommendations and whether the guideline has undergone an external review process. Among the six included guidelines, four guidelines (2015 APA, 2018 NICE, 2018 CANADA, and 2020 EAN) scored >60%. Similar to domain 2, the score of two guidelines (2018 DELPHI and 2018 IPS) was low (26 and 19%, respectively). These guidelines did not address the selection criteria for evidence and were not submitted for external review prior to publication (items 8, 9, and 13).

Clarity and Presentation

Domain 4 "Clarity and presentation" specifies that the recommendations in the guideline are clear and legible. All six guidelines scored >60% in this domain. Furthermore, a high median score (83.5%) in domain 4 suggests that the guideline developers provide clear recommendations.

Applicability

Domain 5 "Applicability" requires the guideline to describe the factors that may facilitate and hinder the application of the guideline, as well as potential resource investment issues. The median score (46.5%) was the lowest among all domains. Of the six included guidelines, three guidelines (2015 APA, 2018 NICE, and 2018 CANADA) scored ≥60%, while the other three scored <60%, and the 2018 DELPHI scored 19%. The main reason for the low score is that this guideline does not consider the potential resource implications of applying the recommendations (item 20).

Editorial Independence

Domain 6 "Editorial independence" prescribes that the guideline should clearly state whether the interest of the funding has influenced the guideline development process and whether there is conflict of interest among the members of the development team. The median score was 79% in this domain, and the scores of five guidelines were >60%. 2018 IPS scored 0% because this guideline did not specify the requirements of this domain (items 22 and 23).

TABLE 3 | Results of inter-rater reliability (ICC) for each guideline.

Included guidelines	ICC	95% CI	F	P
2015 APA	0.807	0.570–0.916	10.922	<0.001
2018 DELPHI	0.920	0.808–0.966	27.398	<0.001
2018 IPS	0.876	0.730–0.946	14.576	<0.001
2018 CANADA	0.828	0.641–0.923	10.737	<0.001
2018 NICE	0.884	0.425–0.964	29.705	<0.001
2020 EAN	0.851	0.679–0.934	11.913	<0.001

CI, confidence interval.

Overall Assessment

Among the six included guidelines, the median score for the six domains ranged from 46.5% to 87.5%, with five domains >60%. The above results showed excellent inter-rater reliability of the guidelines between two reviewers (**Table 3**). Four guidelines (2015 APA, 2018 NICE, 2018 CANADA, and 2020 EAN) met the definition of high-quality CPGs and were used to extract recommendations.

Recommendation Extraction From High-Quality CPGs

The recommendations regarding the management of BPSD were extracted from the four high-quality CPGs (2015 APA, 2018 NICE, 2018 CANADA, and 2020 EAN). First, the content was divided into pharmacological and non-pharmacological treatments and further classified based on common symptoms including psychotic-like symptoms, emotional symptoms, and sleep disorders (see **Supplementary Table 1**). Then, this review sought the consistency of recommendations from **Supplementary Table 1** and formed a recommendation sheet (**Box 1**) based on the latest meta-analysis and other randomized clinical trial results. This sheet contains nine recommendations, of which seven are related to pharmacological treatment and two to non-pharmacological treatment. These recommendations cover the applicability of antipsychotic drugs, medication recommendations, withdrawal times, and several suitable non-pharmacological therapies.

BOX 1 | Summary of the recommendations for the management of BPSD.

- **Pharmacological treatment**

\\ Psychotic-like symptoms

- ✧ When the patients with severe agitation and/or aggressiveness that could probably lead to harming themselves or others, antipsychotic medication is needed, but haloperidol (typical antipsychotics) is not recommended as a priority;
- ✧ When antipsychotics are considered, the choice of antipsychotics should be based on the risk-benefit ratio;
- ✧ When administering antipsychotics, start with a low dose and slowly increase to the lowest effective dose or until unacceptable side effects occur;
- ✧ When administering antipsychotics for 4 to 6 weeks, or tapering the medication for 1-2 weeks, the patients should be regularly evaluated for symptoms;
- ✧ When patients with dementia have been treated with antipsychotic drugs up to 3 months, or no significant clinical response founded after 4 weeks of therapy, antipsychotic medicine should be gradually reduced and discontinued.

\\ Emotional symptoms

- ✧ When patients with dementia have mild to moderate depression, the use of conventional antidepressants is not recommended (unless there are indications for serious mental illness).

\\ Sleep disorder symptoms

- ✧ When patients with dementia are accompanied by insomnia, antipsychotics and melatonin should not be recommended.

- **Non-pharmacological treatment**
  - ✧ When patients with dementia also show agitation/aggressive behaviour, relaxation, social interaction, sensory therapy (e.g. music, aromatherapy), structured activities, and behavioural therapy may be adopted;
  - ✧ When patients with dementia also have insomnia, sleep hygiene education and personalised multi-component sleep management methods e.g. exposure to daylight, exercise and personalised activities could be adopted.

Pharmacological Treatment

Psychotic-Like Symptom Management

Antipsychotics are frequently used for the management of psychotic-like symptoms. In the four high-quality CPGs, applicability of antipsychotic drugs, medication recommendations, and withdrawal times are mentioned. Three guidelines (2015 APA, 2018 NICE, and 2020 EAN) recommend that antipsychotic medication should be used for patients who are severely agitated, aggressive, and/or cause harm to themselves or others. When using antipsychotics, the dose should start at the lowest level and slowly increase to the effective dose or until unacceptable side effects occur. In addition, the risk-benefit ratio of each drug should be considered when selecting an appropriate antipsychotic, as recommended in two guidelines (2015 APA and 2018 NICE).

Presently, both typical and atypical antipsychotics are commonly used for the treatment of psychotic-like symptoms. In the four high-quality CPGs, two guidelines (2015 APA and 2020 EAN) did not endorse the preferred use of haloperidol, a typical antipsychotic. The 2015 APA guideline recommends that in the absence of delirium, haloperidol should not be used as a first-line agent for nonemergency antipsychotic medication treatment. There was a weak recommendation in the 2020 EAN guideline that modern (atypical) antipsychotics can be used instead of haloperidol when pharmacological treatment of agitation/aggressive behavior is necessary. Conversely, risperidone, an atypical antipsychotic drug, is recommended in two guidelines (2018 CANADA and 2020 EAN). The 2018 CANADA guideline recommends that risperidone should be considered if BPSD relapse and antipsychotic treatment needs to be restarted. Moreover, risperidone could be considered as first-line treatment when pharmacological treatment of agitation/aggressive behavior is necessary, as suggested in the 2020 EAN guideline.

Antipsychotics should be considered for reduction or withdrawal in patients using antipsychotics when their symptoms have improved or serious side effects occur. The 2015 APA guideline recommends that dose reduction or discontinuation of antipsychotics should be considered in patients with agitation or psychosis who do not experience a clinically significant response after a 4-week trial of adequate dose of antipsychotics. Additionally, the 2018 CANADA guideline recommends that tapering and discontinuing antipsychotics slowly in collaboration with the patients and caregivers for patients with BPSD treated for at least 3 months, unless the patient experienced a recurrence of symptoms after prior attempts of tapering the antipsychotic medication. Even if symptoms recur, re-attempts to cancel the prescription within 3 months and at least two attempts to discontinue should be considered. Furthermore, periodic evaluation of symptoms is also necessary after initiation of antipsychotic medication, which will determine whether the treatment is effective or there are withdrawal symptoms. During the application of antipsychotics, two guidelines (2018 NICE and 2015 APA) recommend that patients be assessed for symptoms at

least monthly or every 6 weeks to check whether they still require the medication. In the phase of tapering antipsychotics, the 2018 CANADA guideline encourages close monitoring for withdrawal symptoms in patients, especially in those with severe baseline BPSD or long-standing use of antipsychotics, every 1 to 2 weeks. After drug withdrawal, the 2015 APA guideline recommends that evaluation be performed for at least 4 months to identify signs of recurrence.

Management of Emotional Symptom and Sleep Disorder

For the management of emotional symptoms, the use of antidepressants is mentioned in the 2018 NICE guideline; it recommends that antidepressants should not be routinely offered to manage mild to moderate depression in individuals with mild to moderate dementia, unless they are indicated for a pre-existing severe mental health problem.

In the management of sleep disorders, two guidelines (2018 NICE and 2018 CANADA) make recommendations for the use of melatonin and antipsychotic medications, respectively. The 2018 NICE guideline recommends that melatonin should not be offered to manage insomnia in individuals with Alzheimer's disease. The other guideline (2018 CANADA) indicates that the antipsychotic use for the treatment of insomnia should be eliminated based on the lack of evidence for the efficacy of antipsychotics in treating insomnia, potentially harmful side effects, and high cost of treatment.

Non-pharmacological Treatment

Regarding the non-pharmacological treatment of BPSD, two guidelines (2018 CANADA and 2018 NICE) recommend methods for patients with dementia and agitation, mild to moderate depression, or sleep disorders. When patients with dementia show agitation/aggressive behavior, the 2018 CANADA guideline recommends non-pharmacological interventions such as relaxation, social interaction, sensory therapy (music, aromatherapy), structured activities, behavioral therapy, and indicates that attention should be paid to improve the patient's surrounding environment (e.g., light and noise). Meanwhile, the 2018 NICE guideline recommends ensuring that patients with dementia should participate in psychosocial and environmental interventions for distress during antipsychotic treatment and drug discontinuation. When patients with dementia also show emotional symptoms, 2018 NICE recommends that those who have mild to moderate dementia and mild to moderate depression and/or anxiety should receive psychological treatment. When patients with dementia have sleep disorders, 2018 NICE recommends methods such as personalized multicomponent sleep management, including sleep hygiene education; 2018 CANADA also refers to this.

DISCUSSION

Quality Appraisal of CPGs

This study used AGREE II to evaluate the quality of guidelines for the management of BPSD in six domains, i.e., "Scope and purpose", "Stakeholder involvement", "Rigor of development", "Clarity and presentation", "Applicability", and "Editorial independence", and found that the median scores in all domains were >60% except for the "Applicability" domain (**Table 2**). The "Scope and purpose" and "Clarity and presentation" domains had the highest scores at 87.5% and 83.5%, respectively, possibly because all guidelines clearly elaborate on the purpose and explicitly express recommendations related to the management of BPSD. As for the "Editorial independence" domain, most guidelines refer to "what impact the funding institutions had on the development process of the guidelines" in the text, resulting in high scores (79%) in this domain. This suggests that explaining the conflict of interests among the members of the development team will be key to guaranteeing a high score in this domain. Compared with the above three domains, the scores of "Stakeholder involvement" and "Rigor of development" were lower, at 65% and 64% respectively. The reason for the low score in the domain of "Stakeholder involvement" was that two guidelines (2018 IPS and 2020 EAN) scored low in this domain, which may be related to the fact that the guidelines do not mention basic information of the makers and not consider the intentions of the patients. Therefore, a comprehensive description of the information of the makers of the guidelines and the use of questionnaires and other methods to fully consider the intention of the patients will help improve the score in this domain. In the domain of "Rigor of development", two guidelines (2018 DELPHI, 2018 IPS) did not use standard methodological tools to evaluate the literature evidence on which the recommendations were based, resulting in scores <60%. Therefore, the application of a systematic approach will be indispensable for improving the score in the domain of "Rigor of development". For the lowest-scoring domain of "Applicability" (46.5%), three of the six guidelines (2018 DELPHI, 2018 IPS, and 2020 EAN) did not address potential resource investment issues in applying recommendations, suggesting that this domain has not been taken seriously by the compilers of current guideline development processes. Obstacles in guideline implementation are the main considerations for clinicians when applying guidelines. To identify the obstacles that may be encountered in the process of guideline application, guideline developers and users can use assessment tools, such as the "Guideline Implementability Appraisal" instrument. In addition, including multidisciplinary experts will facilitate the guideline application process.

The work of Azermai et al. (5) reported results similar to ours. The domains of "Scope and purpose" and "Clarity of presentation" scored best. It is suggested that guideline makers have generally paid attention to the professionalism of the content covered by the guideline and the clarity of the recommendations in the process of guideline formulation. In addition, the "Editorial independence" domain scored lower in the work of Azermai et al., suggesting that this domain has been given attention in guideline development after 2012. Conversely, the "Applicability" domain had the lowest score in both studies. Therefore, the domain of "Applicability" requires greater attention in the development of high-quality guidelines for the management of BPSD in the future.

Recommendation Extraction From High-Quality CPGs

Pharmacological Treatment

Psychotic-Like Symptoms Management

Among the four high-quality CPGs, the principles of treatment for psychosis-like symptoms were unanimously recognized. All guidelines suggest that the choice of antipsychotics should be based on the individual risk-benefit ratios of each drug. When the patients with severe agitation and/or aggressiveness that could probably lead to harming themselves or others, the use of atypical antipsychotics is recommended. Furthermore, it is recommended that antipsychotics should be started from a low dose and slowly increased to the effective dose by regularly evaluating individual responses for drugs. After receiving antipsychotic treatment, patients with dementia should be considered about discontinuation of antipsychotics drugs, during experience stable symptoms, or present serious side effects, or their symptoms do not improve.

In terms of drug of choice to treat psychotic-like symptoms in patients with dementia, different from the conclusion of systematic review based on behavioral and psychological symptom management guidelines for dementia reported in 2012, haloperidol (typical antipsychotics) is no longer recommended as a priority drug at the suggestion from two guidelines of 2015 APA and 2020 EAN. In Michel's study, haloperidol was also found to be associated with increased mortality in dementia (16). In recent years, atypical antipsychotics, instead of typical ones, have received increasing attention due to their higher efficacy and fewer adverse events. Jin et al. (17) conducted a Bayesian network meta-analysis and found that atypical antipsychotic quetiapine was as effective as haloperidol, but quetiapine with less adverse events than latter one. Among all the atypical antipsychotic drugs for the treatment of psychotic-like symptoms in dementia, risperidone is recommended as the preferred treatment drug in the 2018 CANCDA and 2020 EAN guidelines. These recommendations are supported by the meta-analysis result, due to comparing the safety of atypical antipsychotics for the management of BPSD, showed that among olanzapine, risperidone, and quetiapine, the incidence of somnolence induced by risperidone was the lowest (18).

It is worth noting that the use of antipsychotics should be with a time limitations. Among the recommended suggestions by the three guidelines of 2015 APA, 2018 CANADA and 2018 NICE, antipsychotic medicine should be gradually reduced or discontinued, when patients with dementia have been treated with antipsychotic drugs up to 3 months, or no significant clinical response founded after 4 weeks of therapy. And during the use of antipsychotic medication or when medication is being tapered, the periodic evaluation for symptoms is necessary which will determine whether patients have receiving an effect therapy or show signs of recurring symptoms. More consistent recommendations from four high-quality CPGs selected in this study, the symptoms of patients with dementia treated with atypical antipsychotics should be evaluated every 4–6 weeks at the beginning of treatment with atypical antipsychotic, every 1–2 weeks at drugs reduction phase, and at least 4 months after drugs withdrawal.

Management of Emotional Symptom and Sleep Disorder

Depression is another common clinical symptom in patients with dementia. Among the high-quality guidelines screened in this study, the recommendation in 2018 NICE guideline present against routinely prescribing antidepressants in individuals with mild to moderate dementia. This is an updated and completely contrary to the recommendation of antidepressants in the treatment of depressive symptoms in patients with dementia in the 2012 systematic review (5). The opinions about depression treatment in 2018 NICE guideline were supported by the results from several large randomized, double-blind placebo trials, which antidepressants did not significantly improve depressive symptom, but increase the additional risks in patients with dementia. The cochrane systematic review based on 10 randomized, double-blind placebo trials, showed almost no difference neither in the depression symptom scale nor patients' daily activities and cognitive abilities between the antidepressant group and the placebo group, while more adverse reactions such as dizziness showed in antidepressant treatment than placebo (19). Therefore, antidepressants are not preferential treatment for dementia patients with mild to moderate depression.

Sleep disorders are common clinical problems in dementia, and are associated with significantly increased carer distress and healthcare costs. In term of drug selection for sleep disorders associated with dementia in 2018 NICE guideline, the recommendation against the use of melatonin to manage insomnia in individuals with Alzheimer's disease. McCleery et al. (20) reached similar conclusions by evaluating the efficacy and adverse effects of melatonin, melatonin receptor agonists, trazodone vs. placebo for the treatment of sleep disorders in patients with dementia and showed that melatonin (in doses up to 10 mg) or melatonin receptor agonists were not effective in improving sleep disorders. The 2018 CANADA guideline strongly recommends eliminating antipsychotic use for the treatment of insomnia, such as atypical antipsychotics quetiapine, due to the lack of evidence for the efficacy of antipsychotics in treating insomnia, but rise up the potentially harmful side effects, and high cost of treatment. Therefore, the antipsychotics and melatonin are not recommended attribute to no conclusive evidence for the benefits outweigh the risks.

Non-pharmacological Treatment

Non-pharmacological treatments may be suitable for emotional symptoms and sleep disorders. It is considered highly safe and has the advantage of few adverse reactions compared to pharmacological treatment and it includes relaxation, social interaction, sensory therapy (music, aromatherapy), structured activities, sleep hygiene education, psychotherapy and so on. Two guidelines of 2018 NICE and 2018 CANADA unanimously propose that patients with dementia and sleep disorders should receive sleep hygiene education including avoiding caffeine before bed, regular exercise, managing stress, reducing bedroom noise, etc. And the 2018 NICE guideline recommend that psychotherapy should be considered for dementia patients with mild to moderate depression. Thus, the personalized non-pharmaceutical interventions could be adopted as an alternative

and effective treatments for depression and sleep disorders in patients with dementia.

Limitations

The present systematic review had several limitations. First, the AGREE II tool does not provide clear boundaries to distinguish between high-quality and low-quality CPGs. The high-quality criteria defined in this study were referenced from other literature on similar topics. In addition, this study paid more attention to the quality of the guideline formulation process with the AGREE II tool, in term of recommendations put forward in the guideline, and the applicability of the recommendations was not evaluated with the AGREE REX tool, only supplemented by the latest meta-analysis and randomized controlled trial results. Second, although the management of BPSD was in the scope of all guidelines we investigated, the focus of each CPG was different. Specifically, two guidelines (2018 NICE and 2020 EAN) focus more generally on the management of dementia, and there is less focus on the treatment of BPSD. Two other guidelines are mainly concerned with the use and discontinuation of antipsychotics (2015 APA and 2018 CANADA). Therefore, the recommendations for pharmacological and non-pharmacological treatment of BPSD were not exhaustive across the guidelines. In addition, as this study focused on the quality assessment of the guidelines for BPSD and extracted recommendations based on these guidelines, it inevitably ignored some valuable and reference-significant reviews, and thus could not provide comprehensive recommendations for BPSD of different diseases.

Summary and Outlook

Briefly, strict CPGs can help clinicians provide effective support for the management of BPSD. Based on the AGREE II scores of each field of interest, this review hopes that guideline experts can pay more attention to developing the "Applicability" domain. In addition, as the AGREE II tool is mainly aimed at quality assessment during the guideline development process, to comprehensively screen for high-quality guidelines, the combination of the AGREE II and AGREE REX tools can be considered in the future, where the latter is a supplement to the former and can evaluate the credibility or implementability of guideline recommendations when applied to clinical practice. Future systematic review of CPGs on treatment for BPSD may provide more comprehensive recommendations regarding the treatment, efficacy monitoring, and discontinuation of antipsychotics for BPSD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

HM and XL contributed equally to search data and drafted the manuscript. CW designed the study and revised the manuscription with JL, LJ, and JJ. MZ, QZ, SG, and YC participated in the search for related clinical practice guidelines. AZ, FW, and XZ conducted the literature review.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.799723/full#supplementary-material

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CASE REPORT
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Case Report: Genetic Creutzfeldt–Jakob Disease With a G114V Mutation and One Octapeptide Repeat Deletion as a Mimic of Frontotemporal Dementia

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Genetic Creutzfeldt–Jakob disease (gCJD) characterized by mutations in the prion protein (PrP) gene (*PRNP*) contributes to approximately 10–15% of the overall human prion diseases. Here, we report a rare mutation in the *PRNP* gene in a Han-Chinese family. A 36-year-old man initiated with anxiety and depression followed by progressive dementia, cogwheel-like rigidity combined with tremors, and he was diagnosed with frontotemporal lobar dementia in the first 2 years. The disease progression was relatively slow, and the patient developed into akinetic mutism in 4 years. To characterize the disease, following the pedigree studies, neuropsychological examination, neuroimaging studies, real-time quaking-induced conversion (RT-QuIC) examination, and so on were conducted. We eventually identified a rare mutation of G114V combined with one octapeptide repeats deletion (1-ORPD) in the PrP in the patient by DNA sequencing. In addition, the same mutation and deletion were subsequently identified in the patient's mother without any syndromes. His maternal grandmother had a late onset of the disease in her 60s. Given that 1-OPRD has never been reported in human prion disease before, our first report that both G114V mutation and 1-OPRD appear in the family would forward our understanding of the etiological mechanisms of the gCJD.

Keywords: genetic Creutzfeldt-Jakob disease, prion, PRNP, G114V mutation, one octapeptide repeat deletions

INTRODUCTION

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of rare, fatal neurodegenerative disorders in humans and animals, characterized by the accumulation and aggregation of prions or abnormally folded proteins (1). The abnormally folded proteins PrP^{Sc} have a high number of β-pleated sheets in their posttranslational conformation compared with the

typical α-helices seen in the normal form of the protein (PrP^{c}). The PrP^{Sc} conformation is partially resistant to proteases and acts as a template for further misfolding of the normal PrP^{C} to abnormal PrP^{Sc}. There are three main groups of prion diseases, termed sporadic (Creutzfeldt–Jakob disease [CJD], sporadic fatal insomnia, and variably protease-sensitive prionopathy), genetic (genetic CJD, fatal familial insomnia, and Gerstmann–Straussler–Scheinker syndrome), and acquired (kuru, variant CJD, and iatrogenic CJD). For instance, approximately 85% of CJD cases are sporadic, 10–15% are inherited, and >1% of the cases are acquired (2, 3).

Based on the clinical and pathological features, inherited human prion disease, or genetic prion diseases (gPrDs), are classified as genetic CJD (gCJD) or familial CJD (fCJD), GSS disease, and fatal familial insomnia (FFI). The majority of gCJD have a common feature of rapid progression with a longer survival time; some of them also present as ataxia or Parkinson-like disorders, which have a slower decline over a few to several years (4). The clinical manifestations and neuropathological abnormalities of gPrDs may vary, and the definitive diagnosis of the gCJD requires genetic evidence except for the routine diagnostic elements such as autoimmune encephalitis (5).

The mutation in the prion protein gene (*PRNP*) was identified to contribute to the development of gPrDs. The *PRNP* gene is located at chromosome 20 and encodes prion protein (PrP) with 253 amino acids highly expressed in the central and peripheral nervous systems. The types of mutations in *PRNP* include missense and non-sense mutation, insertions, and deletion (6). Despite that more than 60 *PRNP* variants were identified to be pathogenic to date, five among them, E200K, V210I, V180I, D178N, and P102L account for around 85% of gPrD cases (7).

The G114V *PRNP* variant is a rare subtype in gCJD (8), and only 15 patients from 5 families were reported worldwide. The affected individuals were characterized by an early age of onset, neuropsychiatric symptoms, rapidly progressive dementia, sleep disturbance with predominant pyramidal and extrapyramidal symptoms, and long disease duration (1–5 years) (8–14). Interestingly, a *PRNP* gene variant with one octapeptide repeat deletion (1-OPRD) is highly related to gastric cancer but not prion diseases. In this study, for the first time, we identified a gCJD patient who carries a *PRNP* variant with G114V mutation and 1-OPRD.

METHODS

Pedigree

The pedigree shown in **Figure 1** was provided by the proband's mother (II-8).

Clinical Assessment

The proband (III-10) and one carrier (II-8) underwent systematic neurological and neuropsychological examinations and scoring on the Medical Research Council prion disease rating scale (MRC Scale) (15). This scoring was repeated for the proband and patient II-8 monthly over the telephone in the follow-up studies.

Genetic Analysis

Genomic DNA was extracted from peripheral blood leucocytes of 3 living members in this pedigree using the DNA Isolation Kit (Bioteke, AU1802). Qualified DNA samples were fragmented into 200∼300 bp. The procedure comprises three standard steps: end-repair of fragmented DNA, A-tailing, adapter ligation, and amplification. Hybridization of pooled libraries to the capture probes and removal of non-hybridized library molecules were carried out according to the IDT and xGen Lockdown® Probes (Integrated DNA Technologies). Sample dilution, flow cell loading, and sequencing were performed according to the Illumina specifications. The DNA libraries were sequenced on the HiSeq X10 (Illumina, San Diego, USA) as paired-end 150-bp reads.

Cerebrospinal Fluid Examination

A lumbar puncture was performed on the proband. Cerebrospinal fluid (CSF) 14-3-3 protein was examined at the National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

Real-Time Quaking-Induced Conversion

CSF and skin RT-QuIC were conducted in the proband at the National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

The CSF assay was conducted in a black 96-well, optical-bottomed plate (Nunc, 265301) on a BMG FLUOstar plate reader (BMG LABTECH); 15 μl of each CSF sample was mixed, with 10 μg of recombinant hamster PrP90-231 in a reaction buffer containing 10 mmol/L PBS, 170 mmol/L NaCl, 10 μmol/L thioflavin T (ThT), 10 μmol/L EDTA, and 0.002% SDS in final. The final reaction volume was 100 μl. Each tested sample was quadruplicated. Each reaction contained blank (reaction buffer), negative (2 μl 10% brain homogenate from normal hamster), and positive (2 μl 10% brain homogenate from scrapie agent 263K-infected hamster) controls. The working conditions were as follows: temperature, 55 °C; shaking speed, 700 rpm; shaking/incubation time, 60/60 s; total reaction time, 60 h. ThT fluorescence (450 nm excitation and 480 nm emission) was automatically measured every 45 min as relative fluorescence units (rfu). The cutoff value was set as the average value of the negative controls plus 10 times SD. The sample was considered positive when two or more parallel wells revealed positive reactive curves.

The sites for skin biopsies were behind the right ear. After disinfection with 75% alcohol, the proband received local anesthesia with a subcutaneous injection of 2% lidocaine hydrochloride. A small piece of skin with a size of about 2 × 1 cm^2 was taken with a scalpel by a neurosurgeon. The biopsy skin specimen covered the epidermis, dermis, and adipose tissues; 2% (w/v) of skin homogenate was prepared in lysis buffer (100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 10 mM Tris, pH 7.5). RT-QuIC reaction contained 10 μg of rHaPrP90-231, 1X PBS, 170 mM NaCl, 1 mM EDTA, 0.01 mM ThT, 0.001% SDS, together with 15 μl CSF samples or 2 μl 10^{-2} to 10^{-4} diluted skin homogenates in a final volume of 100 μl. Each sample was assayed in triplicated

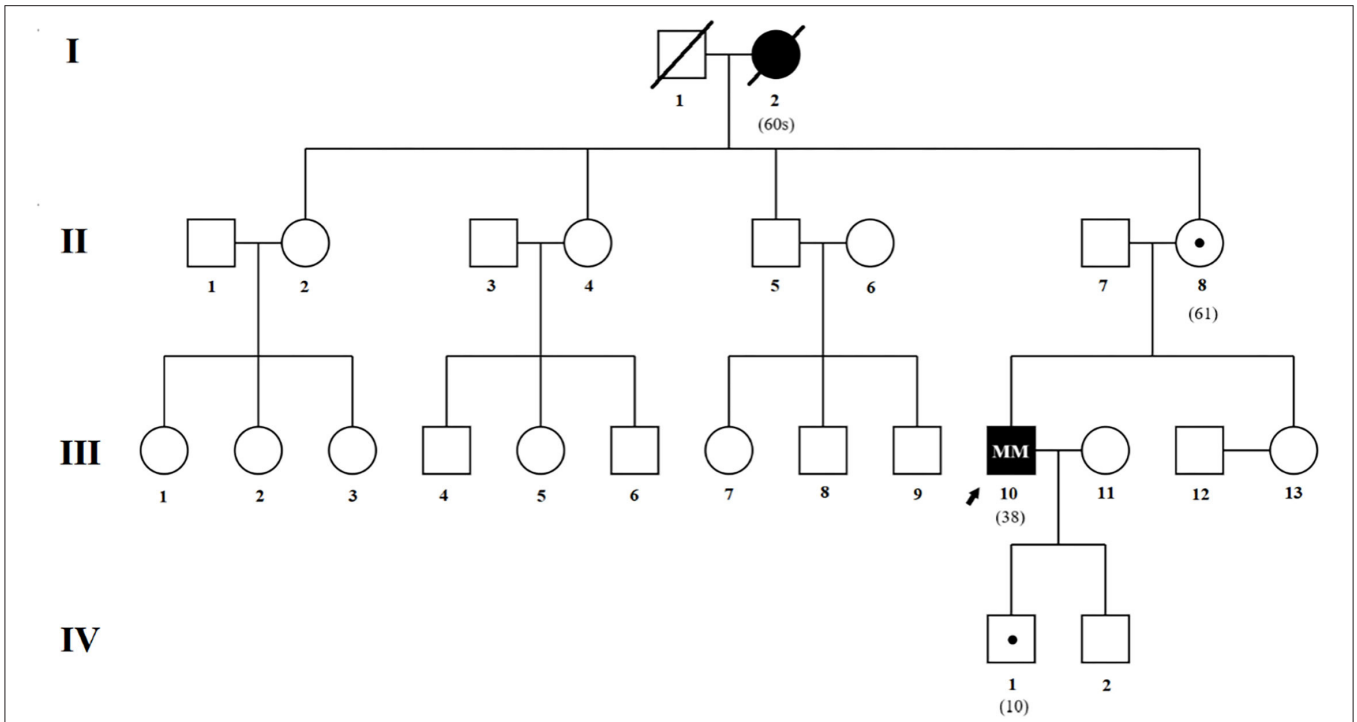

FIGURE 1 | Genealogical tree of the family. Affected patients and asymptomatic carriers are described in the text. Squares indicate males; circles, females; slash marks, deceased; arrow, proband; solid symbols, affected individual; small solid dot with square or circle, asymptomatic carrier.

or quadruplicated. The assay was conducted in a black 96-well, optical-bottomed plate (Nunc, 265301) on a BMG FLUOstar plate reader (BMG LABTECH). The working conditions were optimized as follows: temperature, 55°C; vibration speed, 700 rpm; vibration/incubation time, 60/60 s; total reaction time, 60 h. ThT fluorescence (excitation wavelength, 450 nm; emission wavelength, 480 nm). Each reaction was automatically measured every 45 min and expressed as relative fluorescence units (rfu). The cutoff value was set as the mean value of the negative controls plus 10 times the standard deviation. A sample was considered to be positive when $\geq$2 wells revealed positive reaction curves. The positive control was 10^{-5} diluted the brain homogenate of the scrapie agent 263K-infected hamster, while the negative control was 10^{-5} diluted the brain homogenate of the normal hamster.

Ethical Approval and Consent to Participate

All patients were informed about the purpose of the study and given written consent. The study was approved by the Ethics Committee of the Beijing Tongren Hospital.

RESULTS

Case Report

A 36-year-old Han-Chinese right-handed man (patient III-10, the proband) developed anxiety, depression, sleep disorder, and tremor in his hands after a panic attack but without known medical history. His cognitive state declined, and he was unable to perform job duties due to memory loss. At 11 months after onset, the patient became withdrawn, and more deterioration of his cognitive function was observed, indicated by the difficulty in calculating, being lost at home, and frequently forgetting the names of acquaintances. Besides, the tremors spread to bilateral limbs, which led to difficulty in cake decoration (the patient's profession). After 16 months of onset, he was diagnosed with depression and anxiety and was prescribed sertraline, fluphenazine, and piracetam. At 17 months after onset, he was scored 20 of 30 on the Chinese Mini-Mental Status Examination (MMSE), and 15 of 30 on the Montreal Cognitive Assessment (MoCA Beijing Version). The Hamilton's Depression Scale was 7. At 19 months after onset, he developed hallucinations, which made him see his sons as enemies, and occasionally, he protected himself *via* aggressive behaviors.

In the clinic, the diagnosis of possible behavioral variant Frontotemporal Dementia (bvFTD) was considered because of the abnormal neuropsychological profile, such as early apathy and executive/gene deficits with relative sparing of memory and visuospatial functions.

A thorough neurological examination 20 months after the onset revealed a total deterioration of the cognitive state, dysarthria, slight hypermyotonia, and deep tendon hyperreflexias in the bilateral limbs, and the patient presented cogwheel-like rigidity. He had difficulty finishing the finger-to-nose test and heel-knee-tibia test due to tremors in his limbs. The patient was unsteady when walking on a straight line. No involuntary movement was observed. He scored 15 of 30 on the Chinese

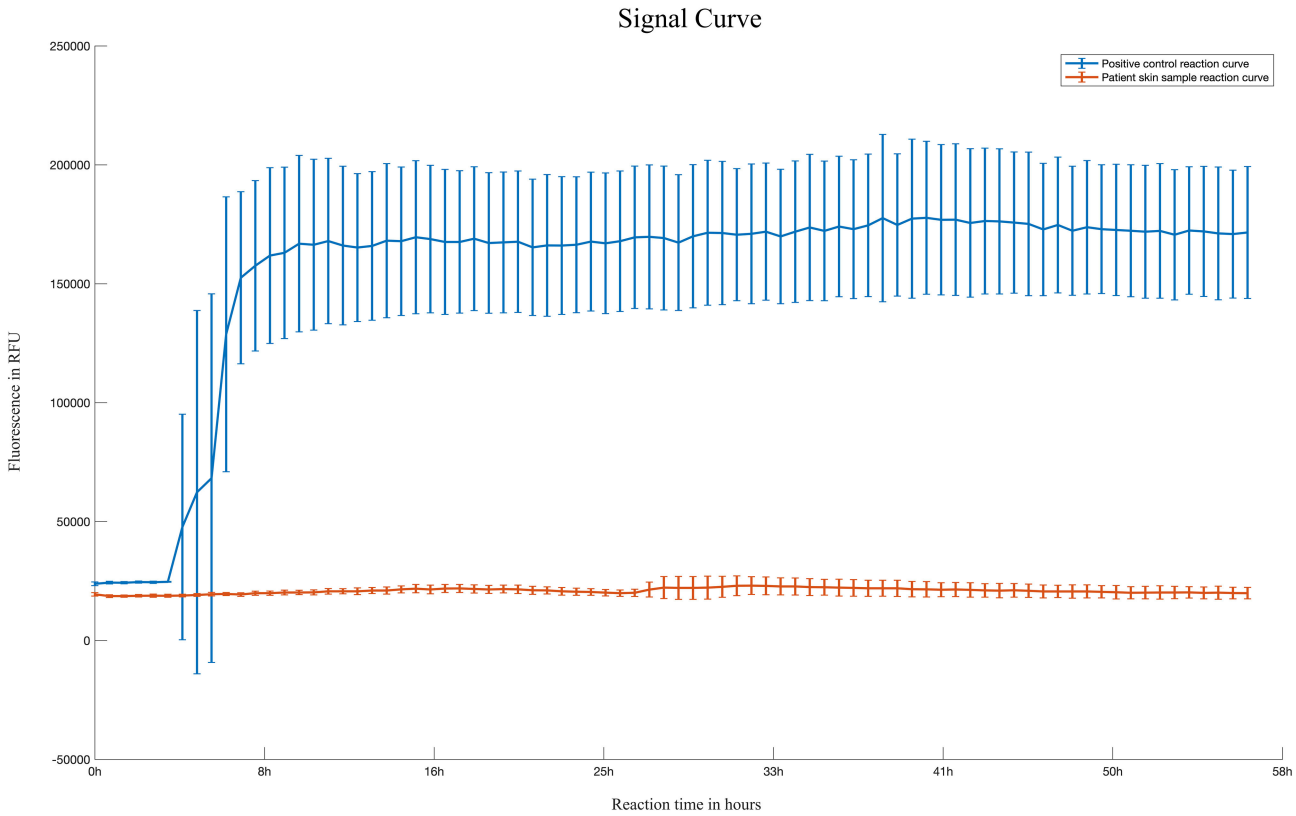

FIGURE 2 | Reaction curve of skin RT-QuIC of the patient. Reaction curves of skin RT-QuIC of the patient. Four replicate reactions of patient skin samples and four replicate reactions of the positive control (hamster scrapie 263K strain brain smear with a 10^{-7} dilution) were used in the RT-QuIC. Each line represents the reaction curve of positive control and patient sample, respectively. The means with standard deviations of those averages are shown as a function of RT-QuIC reaction time. The reaction curve did not show the peak of fluorescence in the patient sample (bottom), indicating a negative result of RT-QuIC. X-axis, hours post-reaction; Y-axis, fluorescence values.

MMSE, and 9 of 30 on the MoCA Beijing Version. The Hamilton's Depression Scale was 7.

To our surprise, the brain MRI demonstrated abnormal intensities in the bilateral caudate nucleus, putamen, and cerebral cortex. A series of laboratory examinations were performed for the rapidly progressive early-onset dementia. The autoimmune screening and tumor marker identification were shown to be unremarkable or negative. An extensive panel for paraneoplastic antibodies including Amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, titin, SOX1, recoverin, zic4, GAD65, and Tr (DNER) were tested, and all were negative. Serology and cerebrospinal fluid (CSF) tests for HIV, cryptococcus, syphilis, tuberculosis, bacteria, fungus, and virus showed no evidence of inflammation. In addition, CSF 14-3-3 protein was tested to be negative. RT-QuIC tests of skin and CSF were also negative (**Figure 2**).

Diffusion-weighted imaging (DWI) sequences displayed restricted diffusion in the bilateral frontal and parietal cortex (**Figures 3A–D**). DWI hyperintensities were revealed in the bilateral basal ganglia, bilateral pulvinar, and dorsomedial thalamus (**Figures 3E–J**). Fluorodeoxyglucose PET (FDG-PET) exhibited hypometabolism in the bilateral cerebral cortex and right basal ganglia (**Figure 4**).

An empiric course of pulse IV gamma globulin was tried without notable improvement.

At 22 months after onset, the hypermyotonia of the patient in the bilateral limbs became more obvious. He had occasional urinary incontinence, and his ability to study was relatively reserved when he scored 21 of 30 on the Chinese MMSE. The MRC Scale score was 13 of 20. The Hamilton's Depression Scale was 6. EEG showed diffuse slow waves. MRI scanning indicated no obvious change compared to images 2 months earlier.

A follow-up study with the MRC Scale revealed gradually developed aphasia, gait disorder, and fecal incontinence 30 months after onset. The patient is still alive, 4 years after the onset, while the MRC Scale score was 2 of 20, and his swallowing function and mobility were still preserved (**Table 1**).

The clinical features indicated that the patient might have prion disease. To determine the etiology of the disease, we extracted genome DNA from peripheral blood leucocytes of the patient and performed a direct DNA sequencing of the *PRNP* coding sequence. Unexpectedly, a rare mutation of G114V and

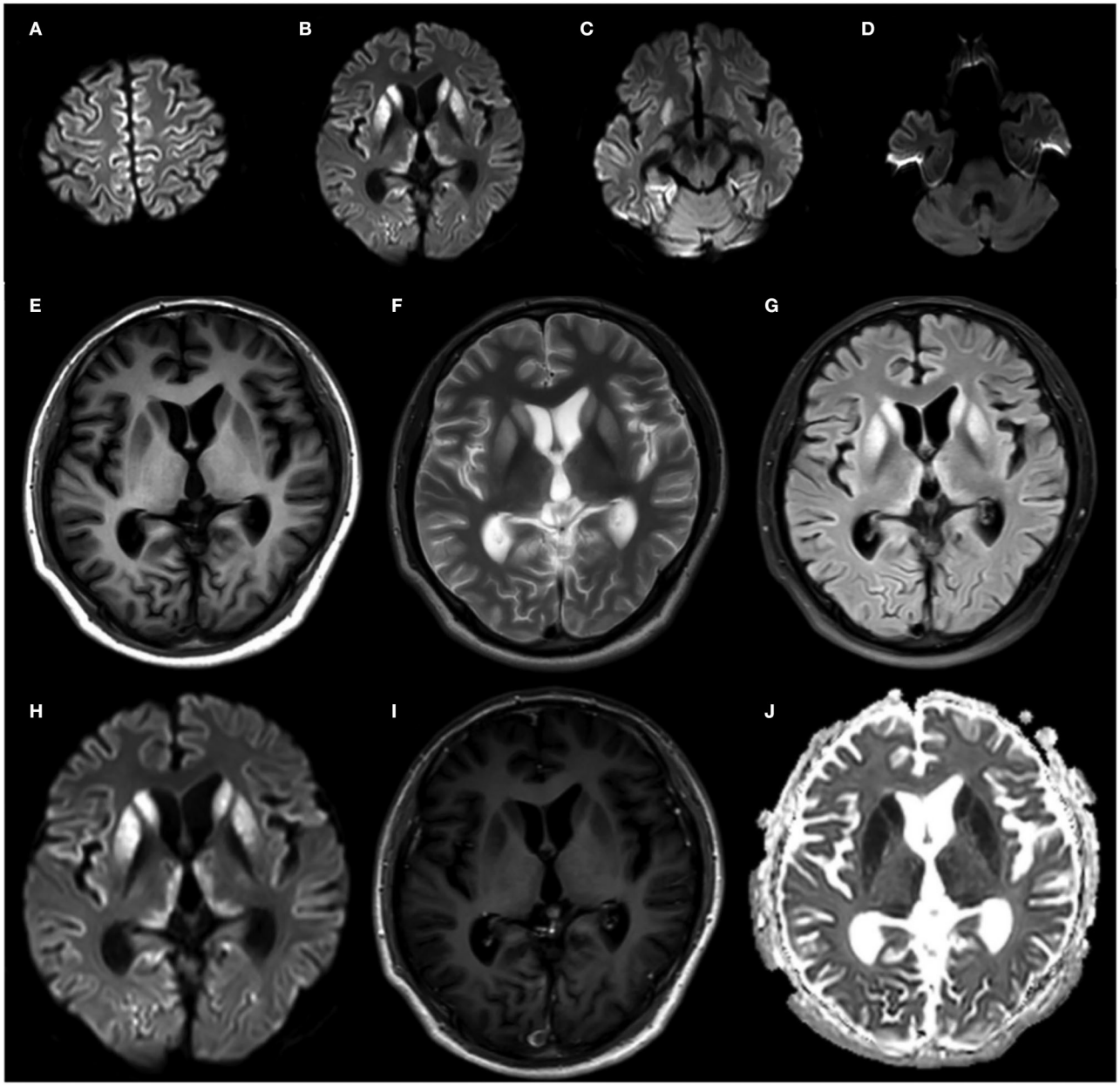

FIGURE 3 | Brain MRI of the proband at 20 months after onset. **(A–D)** DWI sequences displayed restricted diffusion in the bilateral frontal and parietal cortex. **(E–J)** Bilateral basal ganglia hyperintensities, with slight bilateral pulvinar and dorsomedial thalamus hyperintensities on FLAIR **(I)** and DWI **(J)** sequences.

1-OPRD of the PrP in the patient was identified (**Figure 5**). Given that only a few patients with gCJD were reported to carry the G114V *PRNP* variant, we suspected that the mutation in the patient might be inherited from his parents, and we, thus, enrolled his immediate family members in this study for a genetic investigation.

Although the medical record was not available, the maternal grandmother (patient I-2) of the proband was found to have progressive dementia in her 60s, which was within 1 year before her death, and subsequently developed a tremor in her last few months. The proband's mother (carrier II-8) received examination when she was 61 years old, but no neuropsychiatric symptoms were observed. She scored 29 of 30 on the Chinese MMSE and 21 of 30 on the MoCA (Beijing Version). Although the EEG showed slow waves in the left temporal lobe, both cranial MRI (including DWI) and FDG-PET were unremarkable. A follow-up study revealed that she suffered an acute cerebral infarction in the callosum 18 months after the first examination. No cortical ribbon was found by DWI to date (**Table 2**). Whereas DNA sequencing revealed a G114V mutation and 1-OPRD of PrP in this individual. The elder son of the proband (carrier IV-1) received DNA sequencing at age 10, and G114V mutation and

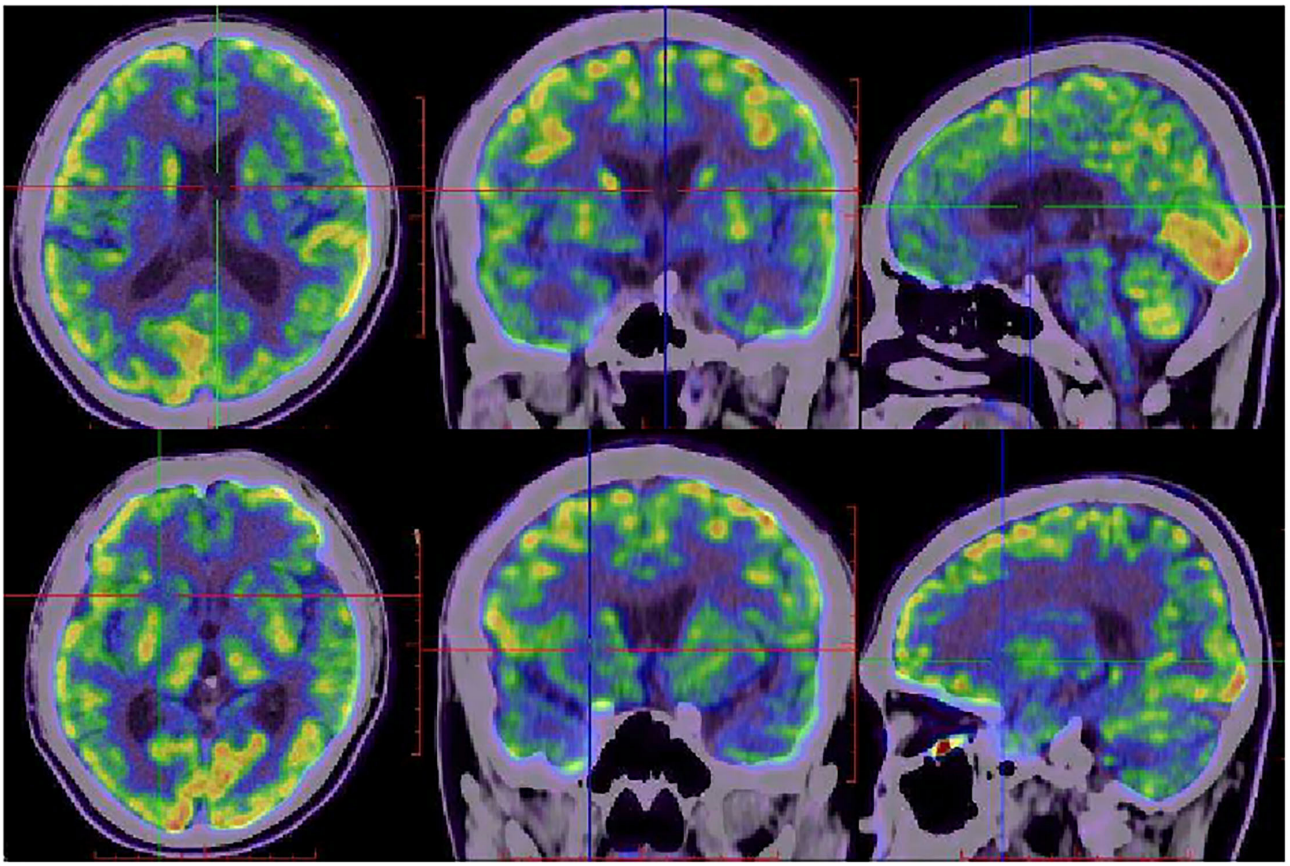

FIGURE 4 | FDG-PET images of the proband at 20 months after onset. FDG-PET showed hypometabolism in bilateral frontal, parietal, temporal lobes, bilateral caudate nucleus, putamen, and thalamus, with right lateralized basal ganglia hypometabolism.

TABLE 1 | The MRC Scale of the proband.

MRC scale	Duration (months)									
	20	22	28	31	34	37	40	43	46	49
Bowel function	1	1	1	0	0	0	0	0	0	0
Bladder function	1	1	1	0	0	0	0	0	0	0
Toilet use	1	1	1	1	1	1	1	0	0	0
Bathing	1	1	1	1	1	0	0	0	0	0
Feeding	2	1	1	1	1	1	1	1	1	1
Transfers and mobility	1	1	1	1	1	1	1	1	1	0
Stairs	1	1	1	1	1	1	0	0	0	0
Best verbal response	3	2	2	1	1	0	0	0	0	0
Memory and orientation to surroundings	2	1	1	1	1	1	0	0	0	0
Judgement and problem solving	1	1	1	1	1	0	0	0	0	0
Use of tools	1	1	1	1	1	0	0	0	0	0
Total	15	13	13	9	9	6	3	2	2	1

1-OPRD were also found, although he did not show any clinical symptoms. No further examination was performed due to his age. No autopsy or biopsy were performed on any of the patients.

Genetic Analysis

Direct sequencing of the *PRNP* coding sequence disclosed a heterozygous missense mutation (**Figure 5A**) at the second position of codon 114, leading to a GGT-to-GTT substitution and a glycine-to-valine change in the PrP (G114V). 1-OPRD was also identified by the sequencing (**Figure 5B**). Both the mutation and deletion were found in 3 family members (**Figure 1**), including patients III-10, and asymptomatic carriers II-8 and IV-1.

Neuropsychological Assessment

At 20 months and 22 months after onset, a series of cognitive investigations were carried out to evaluate the proband's cognitive state (**Table 3**). At 20 months after onset, he scored 15 of 30 on the Chinese MMSE, 9 of 30 on the MoCA Beijing Version, 0 of 3 on the Clock Drawing Test (CDT), 38 of 40 on the Boston Naming Test (BNT), and 3 of 10 on the Copying test. The Hamilton's Depression Scale was 7. The Digit span test (DST) showed 7 in order and 3 in inverted order. The symbol digit modalities test (SDMT) score was 0 of 110. The score of the Rey auditory verbal learning test (RAVLT) exhibited that the ability to learn and recall was significantly reduced. The proband could not finish both the Trail making test (TMT)-A and B. The activity of daily living (ADL) scale and instrumental activities of daily

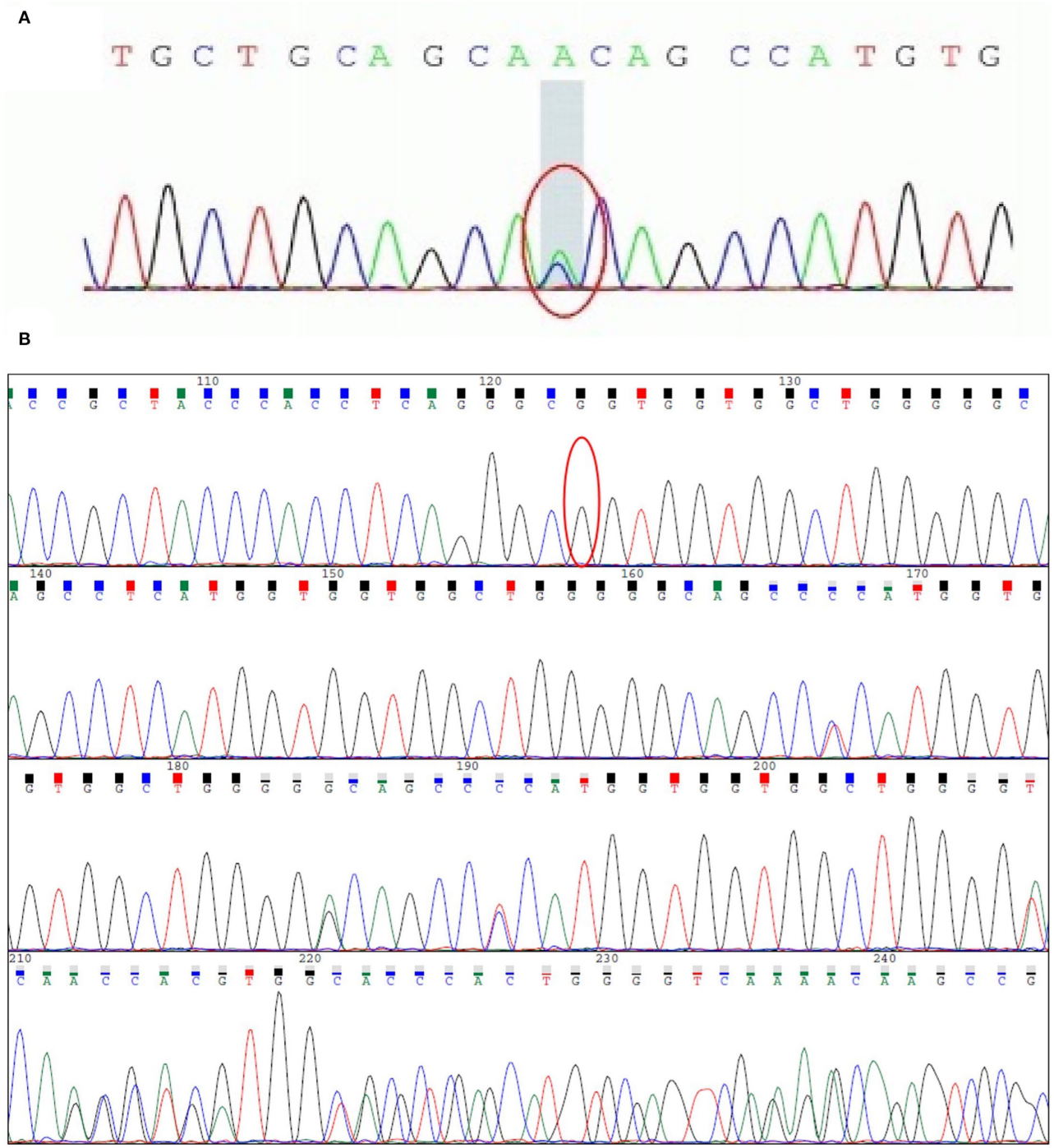

FIGURE 5 | Sequencing analyses in the *PRNP* gene from patient III-10. **(A)** c.341G>T (p.G114V) variant of the PRNP gene was detected. The mutation leads to the alteration of amino acid from glycine-to-valine. This alteration will lead to prion disease. **(B)** c.204_227 delTCATGGTGGTGGCTGGGGGCAGCC (1-OPRD) variant was detected. PRNP gene comprises two exons with the entire open reading frame (ORF) of 762 bp which is contained with exon 2. Nuclides between PRNP codon 51-91 into N-terminal are 5 groups of 24 base pair (bp) repeats, a deletion of the repeat TCATGGTGGTGGCTGGGGGCAGCC was found at the end of the PRNP gene (bottom). This mutation can be found in a minority of people and its pathogenicity has not yet been determined.

living (IADL) scale displayed 15 of 40 and 32 of 40, respectively. Besides, he got 6 on the clinical dementia rating scale sum of boxes (CDR-SB). The MRC Prion Disease Rating Scale (MRC Scale) was scored 15 of 20.

TABLE 2 | Summary of clinical features in affected family members.

Case no.	Sex	Age at onset	Illness of duration, y	Neuropsychiatric symptom	Dementia	Corticospinal signs	Extrapyramidal signs	Ataxia	Myoclonus
III-10	Male	36	4	+	+	-	+	+	+
I-2	Female	60s	<1	NA	+	NA	NA	NA	+

(+), present; (-), absent; NA, not available.

TABLE 3 | Summary of cognitive abilities test in examined family members.

	III-10 (17 months)	III-10 (20 months)	III-10 (22 months)	II-8
MMSE	20	15	21	29
MoCA	15	9	17	21
CDR-SB	NA	6	6	0.5
BNT (20)	NA	19	20	17
CDT	NA	0	1	3
TMT - A, s	NA	NA	240	40
TMT - B, s	NA	NA	NA	NA
DST - in order	NA	7	6	8
DST - inverted order	NA	3	3	3
SDMT	NA	0	0	24
ADL	NA	15	18	10
IADL	NA	32	38	11
Copying	NA	3	3	8
Calculation	NA	3	4	9
MRC Scale	NA	15	13	20

At 22 months after onset, he scored 21 of 30 on the Chinese MMSE, 17 of 30 on the MoCA Beijing Version, 20 of 20 on BNT, 1 of 3 on CDT, and 3 of 10 on the Copying test. DST showed 6 in order and 3 in inverted order. The Hamilton's Depression Scale was 7. SDMT score was 0 of 110. He still got 6 on CDR-SB. The score of RAVLT showed that the patient's ability of learning and recall was increasingly damaged. The TMT-A was finished correctly in 240 s while the TMT-B was not finished. ADL displayed 18 of 40 in PSMS and 38 of 40 in IADL. The score of the MRC Scale was 13 of 20.

DISCUSSION

In this report, we have described a rare G114V mutation and 1-OPRD in PrP in a Chinese family comprising two affected individuals (III-10 and I-2) and two carriers (II-8 and IV-1) (**Figure 1**). II-8 and III-10 were alive at examinations. To the best of our knowledge, our report is the first description of a CJD case with both G114V mutation and 1-OPRD *PRNP* variant.

The proband developed anxiety, depression and progressive dementia, myoclonus, pyramidal, and extrapyramidal syndrome in his late 30s. The DWI sequences in cranial MRI of the patient displayed restricted diffusion in the cortical ribbon, bilateral basal ganglia, bilateral pulvinar, and dorsomedial thalamus hyperintensities. FDG-PET exhibited hypometabolism in the cerebral cortex and the right basal ganglia. The patient was suspected of having CJD while the 14-3-3 protein in CSF was not detected and the RT-QuIC for PrP^{Sc} was negative. Finally, the genetic analysis showed the G114V *PRNP* variant with 1-OPRD.

G114V is a rare mutation linked to the early age of onset, ranging from 18 to 45 years, and long disease duration (1 to 5 years). Since 2005, only 15 patients with G114V-associated genetic prion diseases were identified (the major clinical characteristics are summarized in **Table 4**), and the appearance of G114V mutation seems to have no obvious racial difference. Among the 15 cases, progressive dementia appeared in all cases (100%), extrapyramidal syndromes were reported in 12 cases (80%), and neuropsychiatric symptoms were described in 10 cases (66%). Myoclonus was mentioned in 9 cases (60%) and pyramidal syndromes were noted in 8 cases (53%). Additionally, mild cerebellar signs were reported in a Uruguayan family, and aphasia was described in an American-born Polish descent family (9, 12). Almost all of the patients had neuropsychiatric symptoms and progressive dementia at the onset. In all 15 cases, progressive dementia gradually appeared and lasted the entire clinical course.

The 14-3-3 protein is one of the most common laboratory markers in the diagnosis of CJD for its high sensitivity and specificity. But Muayqil et al. (16) indicate that it can be positive in different neurological diseases, including encephalitis and acute stroke. The 14-3-3 protein was shown to be negative in the proband CSF. Interestingly, CSF 14-3-3 is negative in all reported G114V patients (8–14), which suggests that 14-3-3 protein is not a specific index for this phenotype.

RT-QuIC for PrP^{Sc} in skin and CSF were also negative in the proband, which was not available in other cohorts. These results were first reported in G114V-gCJD. The diagnostic value of RT-QuIC in the detection of sCJD from CSF samples has been demonstrated previously (17). Orrù et al. also reported that the RT-QuIC-based method detects CJD patients with an overall sensitivity and specificity of 100% (18). Besides, according to Xiao et al. skin specimen was ideal for the RT-QuIC test in Chinese patients (19). The RT-QuIC assay was negative in many other gCJDs, such as V180I, V210I mutation (20, 21).

A detailed neuropsychological investigation revealed that the proband was impaired in language and recall and developed time disorientation, attention deficit disorder, dysexecutive syndrome, and visuospatial dysfunction, which is consistent with previous reports. For example, Cousyn et al. reported dysexecutive syndrome and impaired episodic memory with spatial disorientation in their cases (14). A major axis of the frontoparietal dysfunction was also reported in a study by Caine et al. which includes patients with inherited, acquired, and sporadic CJD, suggesting characteristic cognitive features in prominent executive impairment, parietal dysfunction, a largely

TABLE 4 | Features of the 15 reported cases with PrP G114V mutation.

Case no.	Gender	Age at onset (y)	Familiy history	Symptoms at onset	Clinical symptoms								CSF 14-3-3	EEG (PSWC)	MRI	FDG-PET	Genetic analysis	Duration of illness (y)
					Progressive dementia	Personality behavioral changes	Myoclous	Visual hallucination	Cerebellar disturbance	Pyramidal dysfunction	Extrapyramidal dysfunction	Akinetic mutism						
(2005, Rodriguez) Case III-14	Male	27	Positive	Behavior Change	+	+	+	+	-	+	+	+	NA	-	NA	NA	PRNP G114V mutation	2
(2005, Rodriguez) Case III-16	Male	18	Positive	Mood and behavior change	+	+	+	-	-	NA	+	+	NA	-	NA	NA	NA	1
(2005, Rodriguez) Case III-19	Female	22	Positive	behavioral changes, loss of memory, hallucinations	+	+	+	-	+	+	+	-	NA	-	Moderate diffuse encephalic atrophy	NA	PRNP G114V mutation	2
(2005, Rodriguez) Case IV-2	Female	22	Positive	Personality changes	+	+	+	-	+	+	+	+	NA	-	Diffuse cerebral atrophy	NA	PRNP G114V mutation	4
(2005, Rodriguez) Case II-1	Male	28	Positive	Psychiatric symptoms	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2
(2009, Liu) Case III-4	Female	45	Positive	Psychiatric symptoms, progressive dementia	+	+	-	+	-	+	+	-	-	-	Moderate diffuse cerebral atrophy, especially hippocampi bilaterally. DWI hyperintensities in the bilateral caudate nuclei and the putamen, and slight ribbon hyperintensities in the insular cortices.	NA	PRNP G114V mutation	NA
(2009, Liu) Case III-2	Male	45	Positive	Rapidly progressive dementia	+	+	-	-	-	-	+	-	NA	-	Moderate diffuse cerebral atrophy	NA	NA	2
(2009, Liu) Case III-3	Female	35	Positive	Psychiatric symptoms	+	+	-	-	-	-	-	-	NA	NA	NA	NA	NA	2.5
(2009, Liu) Case IV-2	Male	32	Positive	Rapidly progressive dementia	+	-	-	-	-	+	+	-	NA	NA	Normal	NA	PRNP G114V mutation	NA
(2018, Cali) IV-A	Male	24	Positive	Psychiatric symptoms, progressive dementia	+	+	+	+	+	+	+	-	-	-	Restricted diffusion throughout the cortical ribbon, and right, greater than left, cortical atrophy	Decreased but symmetric cortical metabolism, more pronounced in the posterior parietal, temporal, and occipital regions, thalamic uptake was similar to the cortex, and a relative increase in metabolic activity in the basal ganglia.	PRNP G114V mutation	5
(2018, Cali) III-B	Male	32	Positive	Rapidly progressive dementia	+	-	+	+	+	-	+	+	NA	NA	Diffuse cerebral atrophy, markedly out of proportion for age (report only)	NA	NA	2

(Continued)

TABLE 4 | Continued

Case no.	Gender	Age at onset (y)	Family history	Symptoms at onset	Clinical symptoms							CSF 14-3-3	EEG (PSWC)	MRI	FDG-PET	Genetic analysis	Duration of illness (y)
					Progressive dementia	Personality behavioral changes	Myoclous	Visual hallucination	Cerebellar disturbance	Pyramidal dysfunction	Extrapyramidal dysfunction	Akinetic mutism					
(2018, Margolesky) Case	Female	20	Negative	Rapidly progressive dementia, extrapyramidal dysfunction	+	-	+	-	-	+	+	-	NA	-	Abnormal signal in the cortex diffusely and striatum bilaterally	NA	PRNP G114V mutation	NA
(2018, Cousyn) Case	Male	36	Negative	Rapidly progressive dementia	+	-	+	-	-	+	+	-	-	-	Hyperintensities of parieto-occipital cortex	NA	PRNP G114V mutation	4
II-10	Male	36	Positive	Psychiatric symptoms	+	+	+	+	+	-	+	+	-	-	Restricted diffusion in cortical ribbon, bilateral basal ganglia hyperintensities, with bilateral pulvinar and dorsomedial thalamus hyperintensities	Hypometabolism in right basal ganglia	PRNP G114V mutation	5
I-2	Female	60s	Positive	Rapidly progressive dementia	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	<1

expressive dysphasia with reduced motor speed, and it strongly correlated with volume reduction in the frontal and parietal gray matter revealed by MRI (22).

The MRC Scale examination assesses domains of cognitive function, speech, mobility, personal care/feeding, and continence, according to their relative importance documented by the carer's interviews, which can describe the disease progression of a patient. The 20-point functionally oriented scale used in this study is advantageous over single scales for its simplicity of administration and the ability to capture the rapid changes that can characterize the disease. The scores of the MRC Scale for the patient from 20 months to 49 months after disease onset are summarized in **Table 1**, which showed that memory and orientation to surroundings were relatively reserved through long disease duration.

The DWI imaging revealed hyperintensities in the bilateral basal ganglia, pulvinar, and dorsomedial thalamus of the patient. However, hyperintensities in the cortex were restricted to the bilateral frontal and parietal cortex. A similar phenomenon was noted in previously reported one Chinese and two American families carrying the G114V mutation. Compared to another type of gCJD with E200K mutant or sCJD, the ribbon sign is less evident in the cortex in all the G114V patients.

Recently, FDG-PET has been suggested to be used in the diagnosis of CJD, but literature about utilizing FDG-PET in gCJDs is limited (23). In our report, FDG-PET exhibited hypometabolism in the right basal ganglia and cortex of the proband, which is consistent with a previous report that a relative increase in metabolic activity in the basal ganglia as revealed by FDG-PET in a G114V case (12). Interestingly, the basal ganglia and thalamus were unaffected in the context of metabolism of sCJD, as reported previously (24). Thus, measuring the metabolism activity in basal ganglia may help diagnose gCJD.

The EEG of the proband showed diffuse slow waves without typical periodic sharp wave complexes, which are similar to previous reports.

Amino acid 114 is located within a potential membrane-spanning domain of PrP^{C}, which not only belongs to a highly conserved palindromic sequence but also to an amyloidogenic region that is essential for the conversion of PrP (25). G114V has been demonstrated to associate with some pathways, including oxidative phosphorylation, regulation of actin cytoskeleton, MAPK signaling and proteasome, axon guidance, gap junction, and purine metabolism (26). Cali et al. indicated that the conformation of PrP^{Sc} linked to the PRNP-G114V variant might be the principal barrier to transmission (12).

In addition to the G114V mutation, we also detected 1-OPRD in the PrP in the gCJD patient, which has never been reported in the prion diseases. The PRNP gene comprises two exons with the entire open reading frame (ORF) of 762 bp which is contained with exon 2. Nuclides between PRNP codon 51-91 into N-terminal are 5 groups of 24 base pair (bp) repeats, which are also called octapeptide repeats region (OPRP). RNP gene are transmitted in an autosomal dominant manner and include point mutations (such as G114V, E200K, and T188K), insertion of one-nine 24 bp extra repeats or two 24 bp repeats deletion between PRNP codon 51-91 (27, 28).

Insertional mutations of one or more extra octapeptide (from 2 to 12 OR insertions) are associated with CJD (4, 29). Two-octapeptide repeat region (2-OPRD) deletions were reported in two unrelated patients and were considered to be pathogenic (6, 30). The two 2-OPRD cases were characterized by late age of onset (86 and 62 years old) and rapidly progressive dementia. 1-OPRD *PRNP* variant has also been found in healthy individuals with a frequency of less than 1% (31). Although the biological functions of 1-OPRD largely remain unclear, one report suggested that single octapeptide deletion selectively reduces the expression level of pathogenic N-terminal mutants. Besides, 1-OPRD also induces the release of a pathogenic PrP mutant that the mutation occurred within the internal hydrophobic domain from the cell surface (32). 1-OPRD also exists in HeLa cells and several gastric cancer cell lines. Around 66.7% variant frequency in gastric cancer cell lines and 8.9% in gastric cancer tissues were reported, which was significantly higher than that in the normal population (less than 1%) (33, 34). Our report first described 1-OPRD in a familial prion disease, which suggests that 1-OPRD plays a role in the pathogenesis of prion disease.

In summary, we reported a rare pedigree with PrP G114V mutation combined with 1-OPRD, the first report in human gCJD. The proband and his mother and his elder son carry the same mutations. The proband displayed symptoms at the age of 36, but his mother did not develop prion-like disorders in her 60s. The phenomenon would help to forward our understanding of the etiology of gCJD and warrants future study on its pathologic mechanisms.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Beijing Tongren Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XL and YX drafted the initial manuscript and reviewed the literature. XL, YX, and ZZ were in charge of the patients' follow up and cognitive assessments. KX, XC, QS, and XD performed the Skin RT-Quic. JY and HG provide part of imaging. JW and YG revised the manuscript and in charge of all the clinical data analysis, gene analysis, and imaging. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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The Role of Graph Theory in Evaluating Brain Network Alterations in Frontotemporal Dementia

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Frontotemporal dementia (FTD) is a spectrum of clinical syndromes that affects personality, behavior, language, and cognition. The current diagnostic criteria recognize three main clinical subtypes: the behavioral variant of FTD (bvFTD), the semantic variant of primary progressive aphasia (svPPA), and the non-fluent/agrammatic variant of PPA (nfvPPA). Patients with FTD display heterogeneous clinical and neuropsychological features that highly overlap with those presented by psychiatric syndromes and other types of dementia. Moreover, up to now there are no reliable disease biomarkers, which makes the diagnosis of FTD particularly challenging. To overcome this issue, different studies have adopted metrics derived from magnetic resonance imaging (MRI) to characterize structural and functional brain abnormalities. Within this field, a growing body of scientific literature has shown that graph theory analysis applied to MRI data displays unique potentialities in unveiling brain network abnormalities of FTD subtypes. Here, we provide a critical overview of studies that adopted graph theory to examine the topological changes of large-scale brain networks in FTD. Moreover, we also discuss the possible role of information arising from brain network organization in the diagnostic algorithm of FTD-spectrum disorders and in investigating the neural correlates of clinical symptoms and cognitive deficits experienced by patients.

Keywords: frontotemporal dementia, primary progressive aphasia, graph analysis, connectome analysis, small-world, brain networks, magnetic resonance imaging, diffusion tensor imaging

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INTRODUCTION

Frontotemporal dementia (FTD) is a neurodegenerative disorder characterized by executive, behavioral, and/or language deficits (1, 2). The current diagnostic criteria recognize three main FTD subtypes according to clinical presentation: the behavioral variant of FTD (bvFTD), the semantic variant of a primary progressive aphasia (svPPA), and the non-fluent/agrammatic variant of PPA (nfvPPA) (3, 4). bvFTD is the most common subtype characterized by prominent changes in behavior and personality, as well as deficits in executive functions and social cognition (3, 5). On the other hand, loss of semantic knowledge, agrammatism, and fluency deficits are the core features of svPPA and nfvPPA (4).

The highly heterogeneous clinical and neuropsychological phenotype presented by patients with FTD makes the diagnosis of frontotemporal dementia *per se* and FTD subtypes particularly challenging, especially in the early disease stages when the symptoms are more nuanced (1). To overcome this issue several studies have used magnetic resonance imaging (MRI) to identify potential disease biomarkers and help clinicians in establishing a correct and timely diagnosis (6–8). Neuroimaging studies have consistently documented patterns of bilateral fronto-temporal gray matter alterations in patients with bvFTD (9–11). Atrophy in temporal brain regions has been associated with language impairments in patients with svPPA (7, 12), while a higher involvement of frontal regions (i.e., inferior frontal gyrus and insula) is typically observed in patients with nfvPPA (13).

More recently, several studies have applied advanced MRI acquisitions and analyses to obtain an in-depth characterization of brain alterations with respect to the simple gray matter atrophy. Particularly, an increasing number of studies have assessed brain connectivity through graph-theoretical methods, highlighting that this approach shows unique potentialities in FTD (14–29).

Graph theory is an analytical framework that allows describing the brain as a complex network identifying topological properties that reflects global and local information communication (30–33). Global and local graph properties allowed to identify specific patterns of functional and structural alteration in several neuropsychiatric and neurodegenerative disorders, including FTD subtypes (34–38). Moreover, several studies have demonstrated associations between cognitive impairments and network properties, making graph theory a suitable approach to investigate the neural correlates of cognitive performance (34). Nonetheless, graph theory results are often difficult to interpret due to the different metrics and levels (i.e., global and local) at which the analysis can be performed.

Here, we provided a step-by-step guide to interpret graph theory outcomes in FTD. Firstly, we introduced the key concepts underlying brain network construction and described the graph-based properties most frequently used to characterize topological network organization. Second, we provided a critical overview of studies that applied graph analysis in FTD by discussing functional and structural network properties and their association with clinical/neuropsychological variables. Finally, we discussed the pros and cons of graph theory approaches in FTD and points out a future research agenda.

GRAPH THEORY: KEY CONCEPTS AND NETWORK CONSTRUCTION

Network Construction

Graph theory allows modeling a network as a set of discrete elements (nodes) and their mutual relationships (edges) (30, 32, 39). Nodes usually represent predefined brain regions, and edges represent functional or structural connections between regions (30, 31). Two brain regions are considered functionally connected if they display coherent or synchronized neural activity (30, 40). Functionally connectivity is typically estimated using functional MRI (fMRI) (41), but more recent studies have shown that also single-photon emission computerized tomography (SPECT) and F-fluorodeoxyglucose positron emission tomography (FDG-PET) are reliable techniques to assess functional connections (42–44). Structural connectivity is typically estimated by the reconstruction of white matter arising from diffusion tensor imaging (DTI) (45, 46). White matter streamlines can be estimated using deterministic or probabilistic tractography, and several measures of connectivity strength (e.g., number of streamlines, fractional anisotropy, mean diffusivity) can be computed between pairs of brain regions (46, 47). The structural connectivity between brain regions can also be indirectly estimated in terms of covariation of their gray matter morphological properties (volumes, cortical thickness, surface area, and gyrification) or similarity among their gray-level intensity (48–50) based on the assumption that morphological features would covary due to shared axonal connectivity and/or genetic factors (48). For detailed information on the pros, cons, and most appropriate use of each MRI technique, we refer the readers to the study by Islam et al. (51). The defined network is represented through a connection matrix, which is typically filtered by applying thresholding and binarization approaches (52, 53). Different approaches could be used to reduce the influence of spurious connections on network topology, from the simplest application of an absolute or proportional threshold to more recent approaches such as minimum spanning tree (MST) (54). A graphical representation of the framework for the construction of a structural and functional brain network is presented in **Figure 1**.

Segregation and Integration Properties

Different global and local graph metrics are used to assess features of brain network organization. Overall, they can be grouped into information processing integration and segregation metrics (30, 55, 56). Concerning brain network integration, the characteristic path length (L_p) and global efficiency (global_E) are the most frequently used metrics (55–57). L_p is defined as the average shortest path length between all pairs of nodes in the network (56) and global_E is defined as the average inverse shortest path length (57). Brain networks with short L_p and/or high global_E are thought to transfer information across regions more efficiently (52, 56).

The modularity (M) and average clustering coefficient (average_Clust_C) are the two widely used metrics of brain network segregation that allow to assess information processing within specialized brain subsystems (55, 56). M is calculated by partitioning the network into subgroups of nodes maximizing intraconnections and minimizing interconnections (58). The average_Clust_C coefficient is defined as the average fraction in which pairs of neighboring nodes are also neighbors of each other (56). A high value of modularity and/or clustering coefficient mirror a higher propensity of the brain to execute specialized processes within interconnected brain regions (53, 56, 59). A small-world (SW) topology is characterized by high clustering and short path length, which allows to support both segregated/specialized and distributed/integrated information processing (39, 55, 57).

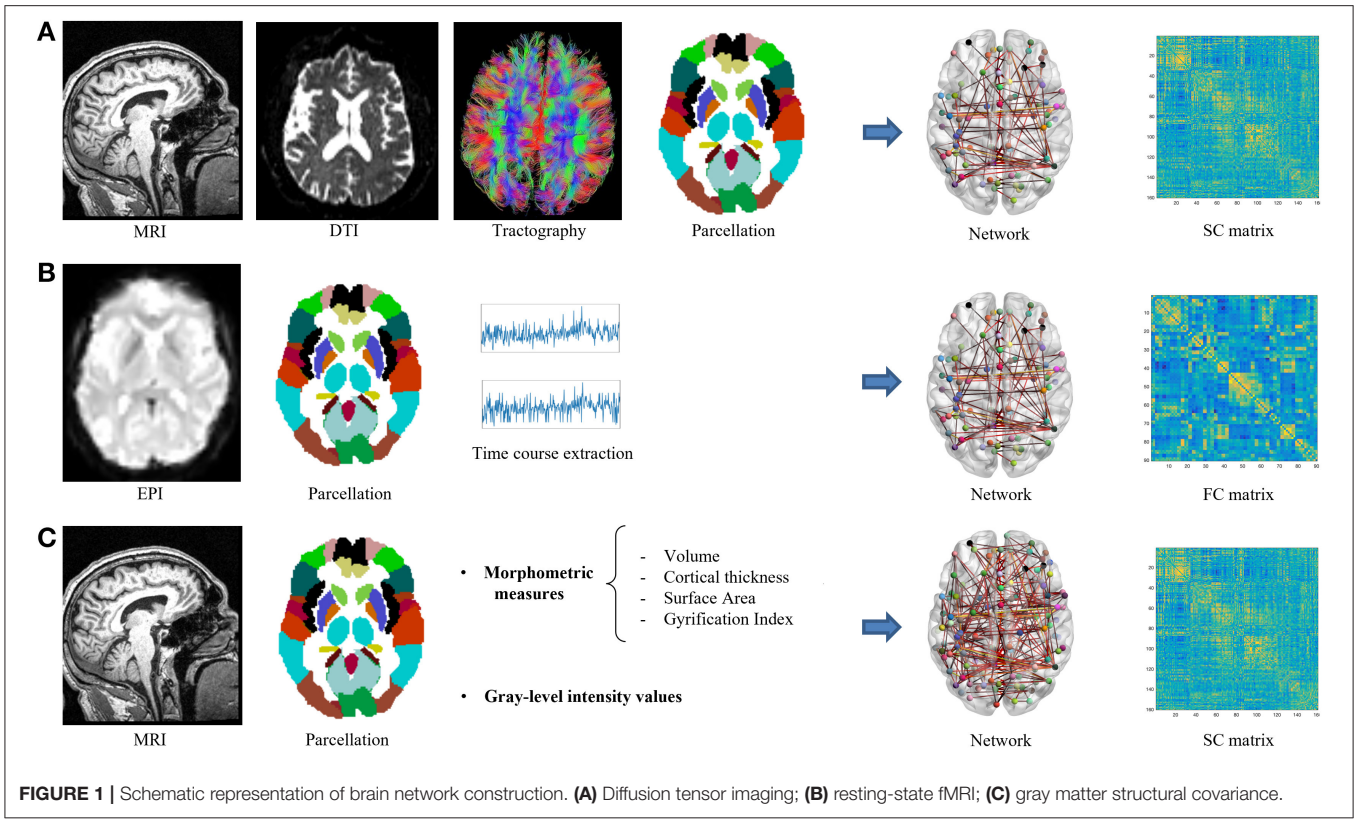

FIGURE 1 | Schematic representation of brain network construction. **(A)** Diffusion tensor imaging; **(B)** resting-state fMRI; **(C)** gray matter structural covariance.

The above-described global metrics can also be defined at a local level to characterize integration (local path-length: local_Lp and local efficiency: local_E) and segregation (local clustering coefficient: local_Clust_C) properties for each brain region (56). Within-module degree and participation coefficient can also be computed for each node to characterize its connectivity within and across modules (58).

Centrality Measures and Hubs Definition

Centrality measures allow to identify nodes with a high influence on the network function (56). Nodal degree (deg) is a measure of centrality defined as the number or the sum of connectivity weights of the edges incident to a node (53, 56, 59). Between centrality (*BC*) measures the fraction of shortest paths between all node pairs in the network that pass through a given index node (56, 59). Closeness centrality (*CC*) measures the mean distance between a given node and the rest of the network (30, 56, 59). Centrality measures allow the identification of network hubs, which represent topologically central regions that play a crucial role in inter-network communication (33). A brain region is usually defined as a hub when its nodal metrics are at least one standard deviation greater than the average of the corresponding measure over the entire network (21, 60). Hub regions tend to be densely interconnected and form a rich-club structure in the brain organization where the hubs are more connected among themselves than to nodes with lower centrality (33).

Regarding networks defined using the MST approach, alternative metrics are used to characterize centrality (maximum degree, maximum betweenness), distance (diameter), and topological aspects (degree divergence, leaf fraction) (54).

NETWORKS ALTERATIONS IN PATIENTS WITH FTD

Sixteen studies applied graph analysis to assess structural and functional brain network alteration in patients with FTD. Eleven studies (68.7%) compared bvFTD patients with healthy controls, one study compared svPPA patients with healthy controls, one study compared nfvPPA with healthy controls and three studies compared FTD subtypes among themselves and with healthy controls. The study from Sedeno et al. reported on a pooled sample of patients with PPA, which did not allow us to discern disease-specific information, therefore, we decided not to consider these results when discussing network alterations of PPA patients. Collectively, these studies analyzed 472 bvFTD, 70 svPPA, 94 nfvPPA, and 15 logopenic-variant primary progressive aphasia (lvPPA) patients. Detailed information for each study is reported in **Table 1**.

Global and Local Networks Alterations in BvFTD

Behavioral variant of FTD is by far the most extensively studied FTD dementia in terms of brain network alterations. Overall, the brain networks of patients with bvFTD showed preserved small-worldness organization, but significant alterations in global properties of the functional network have been consistently observed across studies (14, 17, 18, 23). Studies that applied

TABLE 1 | Summary of studies that used graph analysis in patients with FTD.

Reference	Sample	Mean Age	MMSE	Modality	Network size	Connectivity measures	Binary(B)/ weighted (W)	Global properties	Local properties	Hub (H)/ modularity (M)
Agosta et al. (14)	50 controls 18 bvFTD	61 ± 9 61 ± 8	29 ± 1 21 ± 7	rs-fMRI	90 ROIs grouped into 8 macro-areas	Pearson's correlation	B	Clust_C, Lp global_E, Ass mean deg	deg Bc	H
Agosta et al. (15)	50 controls 13 svPPA	61.0 ± 9.0 59.4 ± 9.6	22.2 ± 7.2 29.0 ± 1.0	rs-fMRI	90 ROIs	Pearson's correlation	B	Clust_C, Lp global_E, Ass mean deg, SW	deg Bc	H
Daianu et al. (16)	37 controls 20 bvFTD 23 EOAD	59.4 ± 9.6 60.7 ± 10.7 59.6 ± 8.8	29.1 ± 0.9 24.1 ± 4.7 23.4 ± 4.2	DTI	68 ROIs	Fiber density FA MD	W	Rich club organization	deg	–
Sedeno et al. (17)	12 controls 14 bvFTD 10 stroke	62.58 ± 6.30 66.42 ± 6.83 54.50 ± 9.80	29.08 ± 1.44 25.50 ± 3.87 28.80 ± 1.09	rs-fMRI	116 ROIs grouped into 7 networks	Wavelet analysis	B	Average Bc	–	–
Sedeno et al. (18)	Site 1: 16 controls 16 bvFTD 13 FIS; Site 2: 29 controls 17 bvFTD 8 PPA; Site 3: 15 Controls 14 bvFTD 15 AD	63.50 ± 7.22 69.37 ± 7.29 62.77 ± 10.4 61.30 ± 7.16 65.23 ± 8.29 60.12 ± 5.81 69.13 ± 6.59 65.33 ± 9.12 64.07 ± 7.34	–	rs-fMRI	90 ROIs	Pearson's correlation	B/W	Lp Clust_C	deg Bc CC	–
Filippi et al. (19)	32 controls 38 bvFTD 37 EOAD	62.3 ± 2.6 63.8 ± 7.3 62.1 ± 3.9	29.3 ± 0.8 22.7 ± 5.8 19.3 ± 4.9	rs-fMRI	220 ROIs grouped into 6 macro-areas	Pearson's correlation	W	Clust_C, Lp local_E mean strength	Clust_C, Lp mean strength local_E	-
Vijverberg et al. (20)	59 bvFTD 90 AD 74 SCD	62.1 ± 6.0 63.1 ± 6.1 61.3 ± 6.6	24.6 ± 3.5 21.1 ± 5.0 28.3 ± 1.9	T1 weighted	90 ROIs	Intra-cortical similarity	B	deg, Lp Clust_C, Bc SW	deg, Lp Clust_C Bc	-
Mandelli et al. (21)	20 controls 20 nfvPPA	68.6 ± 6.0 68.8 ± 7.3	29.1 ± 1.5 26.2 ± 3.7	rs-fMRI	110 regions belonging to the speech production network	Pearson's correlation	–	global_E Lp Ass	deg Bc	H M
Reyes et al. (22)	32 controls 50 bvFTD 14 svPPA 22 nfvPPA	61.25 ± 7.28 65.85 ± 8.1 60.3 ± 7.65 63.63 ± 6.87	28.86 ± 1.27 22.47 ± 6.5 16.67 ± 7.66 16.9 ± 6.92	rs-fMRI	90 ROIs	Pearson's correlation	W	global_E Lp, deg, Clust_C, Bc	–	–

(Continued)

TABLE 1 | Continued

Reference	Sample	Mean Age	MMSE	Modality	Network size	Connectivity measures	Binary(B)/ weighted (W)	Global properties	Local properties	Hub (H)/ modularity (M)
Saba et al. (23)	39 controls 41 bvFTD	61.7 ± 6.5 65.6 ± 7.01	–	rs-fMRI	116 ROIs	Wavelet correlation	B (MST)	Maximum deg, maximum Bc, diameter, Ecc, Ass, deg leaf fraction	–	–
Malpetti et al. (24)	82 controls 82 bvFTD	67.93 ± 6.95 69.37 ± 7.73	68.7 ± 1.5 71.4 ± 2.2	FDG-PET	121 ROIs	Metabolic connectivity	–	–	–	H M
Tao et al. (25)	17 controls 18 nfvPPA 15 lvPPA 9 svPPA	65 ± 8.18 69 ± 5.37 64 ± 8.12 69 ± 5.25	-	rs-fMRI	76 ROIs	Pearson's correlation	B	global_E, Lp Ass, Clust_C SW	Lp Clust_C	H
Zhou et al. (26)	20 controls 64 bvFTD	68.7 ± 1.5 71.8 ± 1.7	29.50 ± 0.1 20.08 ± 4.35	SPECT	90 ROIs	Pearson's correlation	B	global_E SW	local_E Bc deg	H
Nigro et al. (27)	20 controls 25 bvFTD	63.60 ± 5.90 66.92 ± 7.69	27.90 ± 1.68 20.80 ± 5.57	T1	82 ROIs	Joint variation	W	SW	local_E Clust_C deg	-
Ng et al. (29)	47 controls 14 bvFTD 50 AD	63.20 ± 5.00 62.05 ± 5.47 65.45 ± 5.87	29.02 ± 1.15 20.82 ± 5.66 21.21 ± 6.72	rs-fMRI	141 ROIs	Pearson's correlation	W	-	deg, local_E within-module deg partic_c	M
Nigro et al. (28)	110 controls 34 svPPA 34 nfvPPA	63.12 ± 7.49 62.91 ± 6.29 68.32 ± 7.27	29.35 ± 0.77 24.97 ± 5.10 25.54 ± 4.04	T1	82 ROIs	Joint variation	W	SW	local_E Clust_C deg	H

bvFTD, behavioral variant of frontotemporal dementia; svPPA, semantic variant of primary progressive aphasia; nfvPPA, non-fluent/agrammatic variant of primary progressive aphasia; lvPPA, logopenic variant of primary progressive aphasia; PPA, primary progressive aphasia; EOAD, early-onset Alzheimer's disease; FIS, fronto-insular stroke; AD, Alzheimer's disease; SCD, subjective cognitive decline; MMSE, Mini-Mental State Examination; rs-fMRI, resting state functional magnetic resonance imaging; DTI, diffusion tensor imaging; FDG-PET, F-fluorodeoxyglucose positron emission tomography; SPECT, single-photon emission computed tomography; ROI, region of interest; Clust_C, clustering coefficient; Lp, path length; E, efficiency; Ass, assortativity; deg, degree; SW, small-worldness index; Bc, betweenness centrality; Ecc, eccentricity.

graph analysis to resting state-fMRI documented alterations of both integration and segregation of information processing as reflected by lower average clustering coefficient, global efficiency, and higher characteristic path length (14, 18). A recent study that adopted MST-based analysis provided further information documenting a higher diameter and eccentricity (23), which indicates a loss of efficiency in exchange information capacity. Similar results arise from studies that applied graph theory to structural MRI (20, 27), which showed a reduced global efficiency and clustering coefficient, suggesting an overall reduced ability in information transfer. On the other hand, evidence is less conclusive for studies that assessed alterations at the local level. The majority of studies found a reduction of nodal degree, particularly evident over frontal regions (namely, orbitofrontal gyrus, anterior cingulate cortex, superior temporal pole, insula, superior and middle frontal gyri) (14, 16, 17, 19, 26), but alterations have been also observed over the left caudate nucleus, superior parietal and occipital lobes (14). A decreased integration and interconnection in temporal and frontal brain regions were also confirmed by a multicenter study investigating functional brain network organization (18). Moreover, patients with bvFTD showed an extensive reallocation of nodes across modules, most notably in the fronto-parietal, limbic-basal ganglia, and cingulum-temporal modules (24). Studies on structural MRI corroborated these findings by documenting lower local efficiency in the cortical thickness of caudal and rostral middle frontal gyrus, rostral anterior cingulate, and transverse temporal gyrus (27).

Finally, a loss of hubs over different brain regions, namely frontal gyrus (right superior frontal, inferior orbitofrontal gyri, left anterior cingulate cortex, and cuneus), basal ganglia, limbic system, cerebellum, and temporo-occipital cortex has also been reported. By contrast, new hubs appeared in the orbitofrontal and parietotemporal brain regions (14, 24).

Global and Local Networks Alterations in svPPA

The global brain network organization of patients with svPPA was characterized by a decreased global efficiency and clustering coefficient, and a higher characteristic path length (15, 22), which could reflect lower segregation and integration in the overall network organization. This finding was also confirmed by a recent study showing a reduced small-worldness index in the structural brain network of patients (28). At a local level, a reduced nodal efficiency, degree, and clustering coefficient have been observed in several brain regions, including the left middle and superior temporal gyri, entorhinal cortex, amygdala, fusiform, hippocampus, and insula (15, 28). Moreover, a loss of hubs was observed in left-hemisphere regions (15).

Global and Local Networks Alterations in nfvPPA

In patients with nfvPPA, a lower global efficiency was observed over the whole-brain network and in the speech production network (SPN) (21, 22). Increased path length, clustering coefficient, and modularity were also observed in the SPN (21). While the increased path length suggested a reduction in the information integration, the higher clustering coefficient and modularity may indicate a tendency of the network to segregate into smaller communities (21). At a local level, lower clustering coefficient, degree, and local efficiency were observed in several frontal regions including the left caudal and middle frontal gyrus, superior frontal gyrus, and left pars opercularis (27). Moreover, a loss of hubs in the left fronto-parietal-temporal area of the SPN, typically affected by the disease, was also documented while additional hubs were being recruited more anteriorly within the left frontal regions and in the right hemisphere (21).

Global and Local Networks Alterations Between FTD Subtypes

When FTD subtypes were directly compared, a lower global efficiency was observed in patients with nfvPPA relative to bvFTD but not to svPPA (22). Moreover, patients with nfvPPA presented a less small-worldness index than patients with svPPA (28). At local level, significant differences were observed only between PPA subtypes. In particular, decreased clustering coefficient, degree, and local efficiency in the temporal pole were observed in patients with svPPA relative to nfvPPA. By contrast, patients with svPPA display higher values of these local metrics in the left caudal frontal gyrus and left pars opercularis than nfvPPA (28). A different configuration of hubs was also found among PPA variants (25). More in detail, both lvPPA and svPPA showed a lateralized hub distribution (right brain hemisphere) while patients with nfvPPA were characterized by a bilateral distribution across both hemispheres (25).

Association of Brain Network Topology With Clinical/Neuropsychological

A very limited number of studies have correlated graph analysis metrics with clinical/neuropsychological impairments in FTD, with all studies specifically focused on patients with bvFTD.

A lower clustering coefficient in the right hippocampus has been associated with impairment in cognition and executive functioning, while a lower degree in the superior occipital gyrus has been associated with attentional impairments (20). Apathy and inhibition (measured through the frontal system behavior scale) showed a negative association with path length and a positive association with global efficiency, degree, and clustering (22). Increased nodal centrality in the left insular and right frontal hubs resulted associated with the degree of social cognition impairments. More recently, the severity of behavioral alterations (assessed through the neuropsychiatric inventory) was associated with lower modularity in the salience/ventral attention network and higher modularity within the module degree in the left cingulate cortex of the control network (29). Finally, higher overall cognitive functioning (assessed through the MMSE) resulted associated with higher efficiency of caudal anterior cingulate thickness (27).

LIMITATIONS AND FUTURE DIRECTIONS

The diagnosis of FTD-spectrum dementia is established based on clinical presentation, yet at the same time it is becoming increasingly reliant on neuroimaging. Indeed, the current diagnostic criteria (3, 4) require the documentation of frontal and/or anterior temporal atrophy for establishing the diagnosis of "probable" bvFTD. With the advent of new and more sophisticated analytical techniques, such as graph theory analysis and the study of connectome, neuroimaging data are likely to gain a key role in the diagnosis of dementia, including FTD subtypes. However, up to now, graph theory has been extensively applied to document altered brain connectivity in Alzheimer's disease (36, 61–63), while studies in FTD are rare and markedly skewed in favor of bvFTD, with only two studies specifically focused on svPPA and nfvPPA.

In bvFTD, graph analysis revealed a loss of efficiency in the information processing across brain regions reflected by reduced clustering coefficient and increased path length.

The pattern of neuroanatomical involvement highlighted by graph analysis overlapped with that observed in previous studies that analyzed "classic" quantitative neuroimaging metrics (i.e., gray-matter atrophy) in documenting alterations over frontal and temporal regions, further confirming their crucial role in bvFTD pathogenesis (10, 11, 64). Local network alterations showed loss of central nodes in the frontotemporal cortex and limbic system and a reorganization of network hubs, which could either mirror a compensatory process or be related to disease progression. Moreover, global and local metrics were associated with the severity of behavioral symptoms, overall cognitive functioning, and impairment in specific cognitive domains, suggesting that the alterations of information processing may exert a significant effect on the cognitive and behavioral symptoms experienced by patients.

Concerning svPPA, the few available studies documented reduced nodal efficiency, degree and clustering, and loss of hubs over several temporal and limbic regions, which indicates a reduced centrality of these regions in the information transfer. On the other hand, alterations over frontal brain regions such as the caudal middle and superior frontal gyrus were associated with nfvPPA. Moreover, patients with nfvPPA showed a reorganization of hub distribution in the speech production network and loss of hubs in the fronto–parietal–temporal areas.

When network alterations are compared between FTD subtypes, nfvPPA presented a higher impairment of global metrics compared to both bvFTD and svPPA. Moreover, svPPA and nfvPPA showed differences in local metrics: patients with nfvPPA display local abnormalities in brain regions crucial for language production (left caudal frontal gyrus and pars opercularis), while patients with svPPA showed greater impairment in areas associated with language comprehension such as the temporal pole.

Taken together, these results indicate that graph theory is capable of detecting specific brain network alterations in patients with FTD that could potentially serve as a disease biomarker. However, there is a series of methodological issues that limits its broader applicability.

First, there is a lack of standardized protocols for performing graph analysis, resulting in a wide variability of metrics and approaches across studies. Particularly the choice of thresholding, which is often arbitrary, significantly affects graph metric quantification and therefore limits the reproducibility of results. More recent techniques, such as MST, have the potential to overcome this issue but to date have been applied only in one study in the field of FTD.

Second, graph metrics are influenced by the parcellation scheme used to define network nodes, yet no consensus exists regarding which brain parcellation could be considered optimal to capture functional activity or anatomical intersubject variability. Third, all studies reviewed that analyzed fMRI focused on static functional connectivity, assuming temporal stability over scanning time. However, recent studies have reported that connectivity shows time-dependent fluctuations on the scale of seconds to minutes (65). Noteworthy, these time-dependent changes *per se* have provided novel insights into brain organization and should be considered in future studies on patients with FTD (66). Fourth, new reliable and practical frameworks need to be proposed to define graph metrics using the integration of different brain imaging modalities. Finally, all studies applied a "transversal" research design, with different graph metrics being assessed during a singular MRI session, while longitudinal studies are completely lacking, precluding the possibility to quantify the predictive value of these metrics on disease progression.

CONCLUSIONS

Graph analysis is proven to be able to detect specific global and local brain network alterations in patients with bvFTD, while the number of studies is too limited to draw any definitive conclusions on svPPA and nfvPPA. The assessment of network alterations in FTD spectrum may have important clinical implications both in the diagnostic process, as a potential disease biomarker, and in the follow-up as an approach potentially able to track disease course.

AUTHOR CONTRIBUTIONS

Conceptualization: SN and GL. Data curation: BT, RDB, and AC. Investigation: SN, MF, and BT. Methodology: SN, MF, BT, RDB, and AC. Supervision: GL and GG. Writing—review and editing for important intellectual content: SN, MF, BT, AC, GG, and GL. Writing—original manuscript: SN and MF. All authors contributed to the article and approved the submitted version.

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Exome Sequencing of a Portuguese Cohort of Frontotemporal Dementia Patients: Looking Into the ALS-FTD Continuum

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Introduction: Frontotemporal dementia (FTD) is considered to be part of a continuum with amyotrophic lateral sclerosis (ALS). Many genes are associated with both ALS and FTD. Yet, many genes associated with ALS have not been shown to cause FTD. We aimed to study a Portuguese cohort of FTD patients, searching for variants in genes associated with both FTD and/or ALS.

Methods: We included 57 thoroughly characterized index FTD patients from our memory clinic, who were not carriers of pathogenic variants in *GRN, MAPT* or *C9orf72*. We performed exome sequencing and 1) prioritized potential FTD and ALS causing variants by using Exomiser to annotate and filter results; and 2) looked specifically at rare variability in genes associated with FTD (excluding *GRN*, *MAPT* and *C9ORF72*) and/or ALS.

Results: We identified 13 rare missense variants in 10 patients (three patients had two variants) in the following genes: *FUS, OPTN, CCNF, DCTN1, TREM2, ERBB4, ANG, CHRNA4, CHRNB4* and *SETX*. We found an additional frameshift variant on *GLT8D1* in one patient. *One* variant (*ERBB4* p.Arg1112His) gathered enough evidence to be classified as likely pathogenic by the ACMG criteria.

Discussion: We report, for the first time, an expanded study of genes known to cause FTD-ALS, in the Portuguese population. Potentially pathogenic variants in *ERBB4, FUS, SETX, ANG, CHRNA4* and *CHRNB4* were identified in FTD patients. These findings provide additional evidence for the potential role of rare variability in ALS-associated genes in FTD, expanding the genetic spectrum between the two diseases.

Keywords: frontotemporal dementia, ALS (amyotrophic lateral sclerosis), phenotype, cohort, Portuguese

INTRODUCTION

Frontotemporal dementia (FTD) is a clinically, genetically, and pathologically heterogeneous group of disorders that lead to progressive cognitive impairment due to degeneration of the frontal and temporal lobes as a common hallmark. FTD is the second most common cause of early-onset degenerative dementia (1) and about a third of its cases are found to be genetic (2). There are different genes shown to cause this disease, with most of the cases being associated with changes in three genes: progranulin (*GRN*) (3, 4), microtubule associated protein tau (*MAPT*) (5) and chromosome 9 open reading frame 72 (*C9ORF72*) (6, 7). However, the genetic landscape is rapidly expanding, with more and more genes being associated with FTD.

Over time, FTD has evolved to be considered to be part of a continuum with amyotrophic lateral sclerosis (ALS) (8). Much of the support for this association comes from genetic data, as they share many genes as the underlying cause of disease (9), such as *TARDBP, FUS, VCP, TBK1, CHCHD10, SQSTM1, UBQLN2, CCNF, CHMP2B, OPTN, DCTN1, TUBA4A, hnRNP2B1* and *hnRNPA1* (10) and most importantly, the *C9ORF72* repeat expansion (11). Yet, many of the genes that have been associated with ALS have not been shown to cause FTD (11).

In this study, we analyzed a thoroughly characterized Portuguese cohort of 57 patients with FTD. The previous screening of FTD-causative genes (*MAPT*, *GRN* and *C9ORF72*) allowed us to include only cases without mutations in these genes. Here we hypothesize that, given the known genetic and clinical spectrum between FTD and ALS, genes previously only related to ALS or to FTD/ALS may carry rare variants with a role in pure FTD. In this study we search for likely pathogenic variants in genes causative of both FTD (beyond *MAPT*, *GRN* and *C9ORF72*), and/or ALS, through exome sequencing.

METHODS

Subjects

In this study, we included 57 FTD index patients from the memory clinic, in Centro Hospitalar e Universitário de Coimbra, Portugal, a tertiary reference hospital from the central region of Portugal. The diagnosis was performed according to the most widely accepted criteria (12, 13), and supported by extensive characterization. Our clinical protocol included (1) a complete and systematic clinical and neuropsychological evaluation; (2) structural neuroimaging [computed tomography and/or magnetic resonance imaging]; (3) lumbar puncture to determine Alzheimer's Disease (AD) cerebrospinal fluid biomarkers and/or amyloid PET, to strengthen the diagnosis, by excluding AD.

Patients are from a convenience sample recruited between 2012 and 2016. We included 57 patients, 31 of which were female (53.4%). Mean age of onset was 54.5 (34 to 73) years. All patients were from Portuguese ancestry. Of the whole cohort, twenty patients (34.4%) had positive family history (a first-degree relative with a neurodegenerative condition).

The study was approved by the ethics committee of Coimbra University Hospital and biological samples were obtained following written informed from the patients and/or the patients legal representatives.

Exome Sequencing

Exome sequencing was performed for all patients on a HiSeq2500 with 75-100 bp pair-end reads after library preparation with the SureSelect Exome Capture Kit v4 (Agilent). Exome data processing was done following GenomeAnalysisTK best practices. Alignment was done with the Burrows-Wheeler Aligner (bwa-mem) v0.7.12 against the hg19 genome assembly. Duplicates were identified with samblaster v0.1.21 and bases were recalibrated with GenomeAnalysisTK v3.8-1 (14). Variant Quality Score Recalibration (15), and annotation with snpEff v4.2 and dbNSFP v2.9 were applied to all variants (16, 17). Variants were filtered according to their quality metrics as described by Patel and collaborators (18).

We explored variants in genes reported to cause FTD (10): *FUS, TBK1, TARDBP, SQSTM1, CHMP2B, VCP, CHCHD10, UBQLN2, CCNF, OPTN, DCTN1,* and *TUBA4A*. We also explored other genes previously linked to FTD: *TREM2, TYROBP*, *hnRNPA1, hnRNPA2B1* and *TMEM106B*. We further analyzed variants in genes reported to cause ALS (19), namely: *SOD1, ANG, MATR3, FIG4, VAPB, PFN1, SETX, ERBB4, ANXA11, KIF5A, PRPH, GLT8D1, TIA1, DAO, ELP3, TAF15, EWSR1, SS18L1, NEK1, C21orf2, NEFH, CHRNA3, CHRNA4, CHRNB4, PON1, PON2, and PON3*. Genes known to cause ALS in an autosomal recessive pattern and genes associated with copy number variations were not examined. All patients were previously screened for C9ORF72 expansions, GRN and MAPT mutations, and were found to be negative. Variants identified in *CYLD* in this cohort have been reported elsewhere (20).

Only rare variants were included in the study. Variants with a minor allele frequency (MAF) $>$ 0.001 in gnomAD overall sample, or in any of the detailed subpopulations were excluded. *In silico* pathogenicity prediction was assessed using SIFT (Scale-Invariant Feature Transform) (21), Polyphen-2 (22), PROVEAN (Protein Variation Effect Analyzer) (23), Mutation Taster (24) and CADD (Combined Annotation Dependent Depletion) (25). Scores and cut-offs suggested by the software's authors were considered. In CADD, a score higher than 15 was considered as deleterious. The significant findings were comprehensively assessed by considering MAF, predicted pathogenicity, disease association, and family history.

Exomiser Analysis

An adapted version of the Exomiser software (v12.1.0) was used to identify variants that could potentially cause the disease in the individual FTD cases by prioritizing variants related to FTD (HP:0002145) and ALS (HP: 0007354), using an autosomal dominant inheritance model.

The results from the individual runs of Exomiser analyses were filtered by excluding variants without alternative alleles, not passing the Exomiser filter (inheritance), synonymous, with low coverage, and high frequency. Variants that had heterozygous calls in more than three controls and more than 100 cases

were filtered out. Variants that were not present in the available databases were kept and were sorted by the Exomiser Gene Combined score in a descending order. A list was compiled with the three top ranked candidate variants separately for FTD and ALS.

InterVar ACMG Criteria

An adapted version of the InterVar software (v2.2.1) was used to classify variants based on ACMG guidelines (26). Out of the 28 ACMG criteria, InterVar can automatically apply 18 criteria. The variants in **Table 1** were analyzed using the InterVar classification followed by a manual analysis to determine if any of the ten manual criteria were present.

RESULTS

We identified 14 rare variants (13 missense and one frameshift variant) in 11 patients (three patients had two variants), which are reported in **Table 1**.

We found rare missense variants in *FUS*, *OPTN, DCTN1, ERBB4, ANG, TREM2, CCNF, CHRNA4, CHRNB4* and *SETX* and a frameshift variant in *GLT8D1* (p.Lys131fs*7), in one patient.

For several of the analyzed genes we did not identify any relevant variants: *TBK1, TARDBP, VCP*, *TUBA4A, UBQLN2*, *SQSTM1, CHMP2B*, *CHCHD10, hnRNPA1*, *hnRNPA2B1*, *TYROBP* and *TMEM106B*. This was also true for the ALS genes *SOD1, PFN1, VAPB, MATR3, ELP3, TAF15, EWSR1, FIG4, SIGMAR1, NEFH, DAO, KIF5A, TIA1, C21orf2, NEK1, SS18L1, ANXA11, CHRNA3, PRPH*, *PON1, PON2, and PON3*.

The top three results from the Exomiser analysis for each FTD case are presented in **Supplementary Table 1.** None of the variants identified were a clear cause of disease in the respective cases. Some of the top results were identified in the same genes studied here, including *MATR3, TREM2*, *SQSTM1*, and *DCTN1*.

DISCUSSION

Frontotemporal dementia is thought to be part of a spectrum with ALS (8). Although only 10% of ALS cases are considered to be familial (28), the heritability of ALS sums up to 60% (27). Currently, over 25 genes have been associated with ALS (29), but only a fraction of these has also been implicated in FTD. FTD has a strong genetic component but in many familial cases a genetic cause remains to be identified. One may speculate that some of those cases can be attributed to genes which have, so far, been described in ALS cases only.

We studied a sample of 57 FTD Portuguese patients, by performing exome sequencing and (1) prioritizing potential FTD and ALS causing variants by using Exomiser to annotate and filter results; and (2) looking specifically at rare variability in genes associated with FTD (excluding *GRN*, *MAPT* and *C9ORF72*) and/or ALS.

No clearly pathogenic variants were identified in the top results from Exomiser, but some of the prioritized genes overlapped with the genes studied here and known to have a role in FTD and/or ALS. Noteworthy, there was one *GRN* variant (p.Gly70Ser in patient 5), which has been found in controls (30) and one *APP* variant (p.Ala500Thr in patient 18), which has also been found in controls (31).

We identified rare variants in *FUS, OPTN, CCNF* and *DCTN1*, adding support to the role of these genes in FTD. Regarding genes previously only associated with ALS, we identified rare variants in *ERBB4*, *ANG, CHRNA4, CHRNB4, SETX* and *GLT8D1*, some of them predicted to be pathogenic *in silico*. These findings suggest that these ALS genes may also have a role in the pathogenesis of FTD. The absence of significant results in the FTD genes studied here may reflect the strong role of *GRN*, *MAPT* and *C9ORF72* in the disease. Cases with variants predicted to be pathogenic in these genes were excluded from the cohort to increase the probability of finding new genetic factors associated with the disease.

When comparing the phenotypic features between patients harboring rare genetic variants in the ALS genes with those without such variants, we did not see any specific phenotypic differences. However, given the small size of our cohort and the fact that we are considering different genes, such a pattern would be difficult to notice.

Genes Previously Associated With FTD

We identified a novel *OPTN* variant (p.Gln79Arg), predicted to be pathogenic *in silico*, in a male patient with progressive primary aphasia, starting at 62 years old (patient 5). This variant is absent from gnomAD, has a high CADD score (24.7), and leads to a change in the NEMO-like domain of the protein, thought to interact with *TBK1* (32). So far most variants reported in ALS are located at the end of this domain (p.Ala93Pro, p.Lys95Asn, p.Arg96Leu), but there are some variants, namely p.Glu50Lys, which have been shown to be pathogenic, causing normal tension glaucoma (32).

Another variant absent from gnomAD was identified in *CCNF* (p.Glu125Lys) in a female patient diagnosed with bvFTD at age 48 (patient 57). *CCNF* encodes the Cyclin F protein that has been shown to cause abnormal ubiquination and accumulation of ubiquinated proteins, including TDP-43 when mutated (33). This variant is predicted to be pathogenic by SIFT, Polyphen 2 and Mutation Taster and is located between the F-box and the cyclin D regions of the protein (33). Other variants have been described in this region, such as p.Lys97Arg, p.Thr181Ile and p.Ser195Arg (33). These variants have been shown to cause the mislocalization of Cyclin F to the cytoplasm and increased association of Cyclin F with VCP, resulting in activation of VCP ATPase, which plays a positive role in TDP-43 aggregation (34).

For both variants, in *OPTN* and *CCNF* the *in silico* analyses performed pointed to potential deleterious effects. In addition, the absence of these variants from gnomAD supports a potential pathogenic role in disease. Still, these variants had uncertain significance when using the ACMG guidelines and further evidence would need to be found to support their pathogenicity.

Other rare variants were identified in *DCTN1*, *FUS* and *TREM2*, but contrary to the ones described above, these are all reported in gnomAD.

The *DCTN1* variant (p.Arg409Trp), although rare and predicted to be pathogenic *in silico*, is located outside the cytoskeleton-associated protein-glycine-rich domain of the

TABLE 1 | Rare variants identified in genes previously associated with Frontotemporal and/or Amyotrophic Lateral Sclerosis in the Portuguese cohort of Frontotemporal patients.

Gene	Genomic position (hg19)	Zigosity	Variant	Patient	rs	gnomAD MAF*	CADD	PROVEAN	SIFT	Polyphen2	Mutation Taster	Additional variant	ACMG criteria	ACMG classification
OPTN	10:13152343[a] > G	Het	p.Gln79Arg	Patient 5	-	0.000	24.7	−2.045 (N)	0.007	0.993 (D)	0.996821 (PD)		PM2 + PP3	Unc Sig
CCNF	16:2487156G > A	Het	p.Glu125Lys	Patient 57	-	0.000	23.3	−1.035 (N)	0.010 (P)	0.554 (PD)	1.000(P)		PM2 + PP3	Unc Sig
DCTN1	2:74597375G > A	Het	p.Arg409Trp	Patient 51	rs150368544	0.00001761	27.5	−4.761 (D)	0.0 (P)	0.913 (D)	0.99239 (P)		PM1 + PM2 + PP3	Unc Sig
FUS	16:31201719C > T	Het	p.Pro432Leu	Patient 35	rs773104641	0.0001497	7.163	−5.95 (D)	0.001 (D)	1.000 (D)	1.000 (P)	*SETX* p.Thr372Pro	PM1 + PM2 + PP3	Unc Sig
TREM2	6:41129275G > C	Het	p.Asp39Glu	Patient 10	rs200392967	0.0001259	23.8	−1.254 (N)	0.13(T)	0.246 (B)	0.91608 (Pm)		PM2	Unc Sig
ERBB4	2:212251724C > T	Het	p.Arg1112His	Patient 23	rs770460785	0.00002324	23.0	−1.566 (N)	0.032	0.003 (B)	1.000 (P)	*CHRNB4* p.Val220Met	PM1 + PP2 + PP2 + PP3	Likely Pathogenic
ANG	14:21162091G > C	Het	p.Gly123Ala	Patient 32	-	0.00006193	0.06	−1.233 (N)	0.1	0.048 (B)	1.000 (Pm)		PM1 + PM2 + BS4	Unc Sig
SETX	9:135205871T > G	Het	p.Thr372Pro	Patient 34	rs145145045	0.00002328	22.4	−0.962 (N)	0.002	0.073	0.99786 (Pm)	FUS p.Pro431Leu	PM2	Unc Sig
SETX	9:135202313T > C	Het	p.Thr1558Ala	Patient 16	rs764920626	0.000008822	0.002	−0.233 (N)	0.243	0.015	1.000 (Pm)	*CHRNA4* p.Thr545Met	PM2 + BP4	Unc Sig
SETX	9:135211763G > A	Het	p.Ser213Phe	Patient 13	rs1254442456	0.000	28.1	−2.093 (N)	0.0	0.998 (D)	0.87885 (P)		PM2 + PP3	Unc Sig
CHRNA4	20:61981129G > A	Het	p.Thr545Met	Patient 16	rs121912282	0.00007112	2.412	−0.01 (N)	0.124 (T)	0.862 (D)	1.000 (Pm)	*SETX* p.Thr1558Ala	PM1 + PM2 + BP4	Unc Sig
CHRNB4	15:78927807G > A	Het	p.Leu60Phe	Patient 29	-	0.000	25.1	−2.95 (D)	0.001 (D)	0.726 (D)	0.82638 (P)		PM1 + PM2 + PP3	Unc Sig
CHRNB4	15:78921989C > T	Het	p.Val220Met	Patient 23	rs774714066	0.0001161	20.6	−0.62 (N)	0.101 (T)	0.999 (D)	0.9995 (P)	*ERBB4 p.Arg1112His*	PM1 + PM2 + PP3	Unc Sig
GLT8D1	3:52730611 GT > G	Het	p.Lys131fs	Patient 41	rs1308367710	0.000		NA	NA				PM2 + PM4 + BS4	Unc Sig

**gnomAD MAFs correspond to non-Finnish Europeans. Pt, Patient; Het, heterozygous; rs, reference SNP; MAF, Minor Allele Frequency; ACMG, American College of Medical Genetics (27); CADD, Combined Annotation Dependent Depletion; PROVEAN, Protein Variation Effect analyzer; SIFT, Scale-Invariant Feature Transform; Unc Sig(28), Unclear Significance; PM, Pathogenic Moderate [number according to ACMG guidelines (27)]; PP, Pathogenic Supporting [number according to ACMG guidelines (27)]; BP, Benign Supporting [number according to ACMG guidelines (27)]; N: Neutral; D: Damaging, T: Tolerable; P: Pathogenic; B: Benign; PD: Probably Damaging; Pm: Polymorphism.*

protein, where all the variants reported to date and considered to be pathogenic are located (35). This was found in another female patient with bvFTD, starting at the age of 48 (patient 51). This variant was also determined to be of uncertain significance by the ACMG guidelines and further evidence would be needed to support its pathogenicity.

FUS mutations in pure FTD are rare (36). Here, one female bvFTD patient with an age at onset of 53 years (patient 34) carried a missense variant (Pro432Leu), predicted to be pathogenic *in silico*. This variant has been previously associated with familial essential tremor (37). The proline at position 432 is highly conserved and located in the zinc finger domain. It may affect splicing as it affects the last nucleotide of exon 12. However, no other variants in this domain have been associated with ALS or FTD (36) and this was found to be of uncertain significance by the ACMG guidelines. This patient also carried a rare *SETX* variant (**Table 1**).

In *TREM2*, we identified an heterozygous missense variant (p.Asp39Glu), previously described and of unclear pathogenicity in FTD (38). This variant was found in a female patient with a primary progressive aphasia variant, starting at the age of 61 (patient 10).

Variants in Genes Associated Only With ALS

One of the main aims of this study was to search for rare, potentially pathogenic, variants in genes associated with ALS with no previous association with FTD. Specifically looking into these genes, we identified rare variants in *ERBB4*, *ANG*, *SETX*, *CHRNA4*, *CHRNB4* and *GLT8D1*, the latter being a frameshift variant present in one patient.

Recently, a variant in *ERBB4* has been described in one FTD-ALS patient (39), supporting the idea that genes associated with ALS may be also implicated in FTD pathogenesis. However, the assessment of cognitive impairment in that patient was complicated by dysarthria and no clear frontal impairment was documented in the study. We report a variant (p.Arg1112His, in patient 23), which is predicted to be pathogenic *in silico* by SIFT and Mutation Taster. This, together with the previously described ALS-FTD case, gives some support to the potential for this gene to be involved in FTD. This variant was classified as likely pathogenic by the ACMG guidelines.

Angiogenin (*ANG*) is a pro-angiogenic and neurotrophic factor with an important role in stress-induced injury, that acts by promoting neovascularization and neuronal survival (40). Pathogenic variants in this gene have been implicated in ALS (41). Interestingly, in an ALS-FTD cohort, the levels of angiogenin in CSF were only elevated in patients with FTD (40) and at least one patient reported in the literature seemed to have ALS-FTD (42). The variant we identified (p.Gly123Ala) is located close to the previously reported variants p.Arg121His and p.Arg122His (43, 44). The patient carrying this variant is part of a family with 3 of 4 siblings affected, and this variant was only present in two of them. It was also present in one descendant, currently not affected. This, together with the *in silico* data, argues against the pathogenicity of this variant, or, less likely, the presence of a phenocopy in the family.

Senataxin (*SETX)* is associated with juvenile ALS in an autosomal dominant inheritance pattern and with ataxia with oculomotor apraxia type 2 in a recessive inheritance pattern. Senataxin is a protein associated with R-loop regulation. An R-loop forms when an RNA transcript displaces homologous DNA sequence of one of the DNA strands of chromosomes and hybridizes to the complementary sequence of the other strand, generating a short region of RNA-DNA hybrid and single stranded DNA. R-loop dysfunction is increasingly recognized as the cause of several disorders, such as *C9orf72* associated ALS (45). In our cohort, we identified three rare *SETX* variants. One patient carried a variant (p.Thr1558Ala, in patient 16) close to a previously reported pathogenic variant (p.Thr1863Ala) (46). Other rare variant identified in this Portuguese cohort was p.Thr372Pro (patient 34), located in the helicase domain. The third variant was p.Ser213Phe, the only one predicted to be deleterious. This variant is also not reported in gnomAD. This variant was found in a male patient with bvFTD starting at the age of 55 (patient 13).

CHRNA3, CHRNA4 and *CHRNB4* have been associated with ALS (47). These genes encode neuronal nicotinic acetylcholine receptors and regulate $Ca2^{+}$ influx to the neurons. Rare missense variants in the intracellular domains of these proteins have been shown to be more prevalent in sporadic ALS patients (48). They also have been shown to be associated with cognitive performance (49). In particular, *CHRNB4* has been linked to the modulation of attention in attention deficit/hyperactivity disorder (50). Together, these data point to the possible role of variability in FTD risk and/or pathogenesis. We identified three rare variants in this gene cluster, with two variants located in *CHRNB4* (p.Leu60Phe, in patient 29, and p.Val220Met, in patient 23) being particularly relevant, as they are very rare or absent in gnomAD and predicted to be pathogenic *in silico* by five of five (p.Leu60Phe) and three of five (p.Val220Met) tools (**Table 1**). However, all three of these variants were classified with uncertain significance by the ACMG guidelines.

Finally, we found one female patient (patient 42) with an age at onset of FTD at 66 years carrying a frameshift variant in *GLT8D1*, a gene recently associated with ALS (51). It encodes a glycosyltransferase enzyme of unknown function, possibly linked to ganglioside synthesis. *GLT8D1* has also been linked with schizophrenia (52, 53). So far, the vast majority of variants reported to cause ALS are missense and located in exon 4 (51). One variant in exon 10 has also been described in a Chinese cohort (47). The variant identified in our cohort (which is not present in gnomAD) leads to a change in the same glycosyltransferase domain of the protein but is predicted to cause a premature stop codon. It is unclear if variants causing a truncated protein will have the same functional effect as the missense variants previously identified in ALS. However, given that in this case we were able to assess the sister of the proband that also presented with FTD-ALS and did not carry the variant, the likelihood of pathogenicity for this variant is very low.

This study has some limitations, including the small size of the cohort, the lack of pathological diagnoses and the unavailability of samples from family members to test co-segregation of variants with disease. Only one of the FTD patients had a pathological confirmation of diagnosis, although all patients in the cohort were clinically evaluated and thoroughly studied in an experienced dementia focused centre. Due to the absence of family history or the unavailability of samples from family members we did not perform segregation analyses to further study the pathogenicity of most of the reported variants. However, the frequency of the variants, together with the use of *in silico* prediction tools and functional annotation allowed us to weight the possible role of the identified variants.

This study described for the first time the genetic variability beyond *GRN, MAPT* and *C9ORF72*, in a clinically well characterized cohort of FTD cases from Portugal. The identification of rare variants in *FUS, SETX*, *CHRNB4*, *CHRNA4* and *ERBB4* in FTD patients provides additional support for a role of ALS-associated genes in pure FTD cases. This expands the spectrum between FTD and ALS on a genetic level. Replication and extension of these findings can have a significant impact on the genetic counseling of affected patients and families and on the understanding of FTD mechanisms.

DATA AVAILABILITY STATEMENT

The variants identified in this study have been submitted into online repositories. The name of the repository and accession numbers can be found below: National Center for Biotechnology Information (NCBI) ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/, VCV001344510-VCV001344518, VCV000586548, VCV000648503, VCV000905893, VCV000037069, and VCV000098320.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Coimbra University Hospital, Coimbra, Portugal. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MT-P has drafted the manuscript and prepared the final version. MT-P, RG, and JB have designed the study. IS, MT-P, IB, and MA have contributed in the collection of samples and patients study. MT-P, EG, KP, MA, RG, and JB have contributed to the analysis of the bioinformatic data. MT-P, IS, KP, EG, RG, and JB have reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Molecular imaging biomarkers in familial frontotemporal lobar degeneration: Progress and prospects

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Familial frontotemporal lobar degeneration (FTLD) is a pathologically heterogeneous group of neurodegenerative diseases with diverse genotypes and clinical phenotypes. Three major mutations were reported in patients with familial FTLD, namely, progranulin (*GRN*), microtubule-associated protein tau (*MAPT*), and the chromosome 9 open reading frame 72 (*C9orf72*) repeat expansion, which could cause neurodegenerative pathological changes years before symptom onset. Noninvasive quantitative molecular imaging with PET or single-photon emission CT (SPECT) allows for selective visualization of the molecular targets *in vivo* to investigate brain metabolism, perfusion, neuroinflammation, and pathophysiological changes. There was increasing evidence that several molecular imaging biomarkers tend to serve as biomarkers to reveal the early brain abnormalities in familial FTLD. Tau-PET with ^{18}F-flortaucipir and ^{11}C-PBB3 demonstrated the elevated tau position in patients with FTLD and also showed the ability to differentiate patterns among the different subtypes of the mutations in familial FTLD. Furthermore, dopamine transporter imaging with the ^{11}C-DOPA and ^{11}C-CFT in PET and the ^{123}I-FP-CIT in SPECT revealed the loss of dopaminergic neurons in the asymptomatic and symptomatic patients of familial FTLD. In addition, PET imaging with the ^{11}C-MP4A has demonstrated reduced acetylcholinesterase (AChE) activity in patients with FTLD, while PET with the ^{11}C-DAA1106 and ^{11}C-PK11195 revealed an increased level of microglial activation associated with neuroinflammation even before the onset of symptoms in familial FTLD. ^{18}F-fluorodeoxyglucose (FDG)-PET indicated hypometabolism in FTLD with different mutations preceded the atrophy on MRI. Identifying molecular imaging biomarkers for familial FTLD is important for the *in-vivo* assessment of underlying pathophysiological changes with disease progression and future disease-modifying therapy. We review the recent progress of molecular imaging in familial FTLD with focused on the possible implication of these techniques and their prospects in specific mutation types.

KEYWORDS

familial frontotemporal lobar degeneration, molecular imaging, biomarkers, MAPT, GRN, C9orf72

Introduction

Frontotemporal lobar degeneration (FTLD) encompasses a set of clinical syndromes characterized by progressive abnormalities in behavior, executive function, language, or motor function. Patients with FTLD may present clinical syndromes with the behavioral variant of frontotemporal dementia (bvFTD), the nonfluent variant of a primary progressive aphasia (nfvPPA), a semantic variant of PPA (svPPA), and some patients also have amyotrophic lateral sclerosis (ALS), corticobasal syndrome (CBS) or progressive supranuclear palsy (PSP) (1). Approximately, 40% of patients with FTLD have a positive family history of autosomal dominant inheritance (2). Three major mutations were reported in patients with familial FTLD, namely, the microtubule-associated protein tau (*MAPT*), progranulin (*GRN*), and the repeat expansions in the chromosome 9 open reading frame 72 (*C9orf72*). These mutations could lead to neurodegenerative pathological changes years before symptom onset (2, 3).

Mutations in the *MAPT* gene located on chromosome 17q21 first reported in 1998 (4) were discovered in numerous pedigrees of familial FTLD. The majority of known mutations in the coding region occur in the repeats, causing the decreased ability of tau proteins to interact with microtubules, and resulting in hyperphosphorylated tau accumulation in neurons and glial cells (5). *MAPT* mutations of different subtypes have been linked to various tauopathies. Generally, the mutations inside exon 10 (i.e., N279K, S305N, P301L) and intron 10 (i.e., IVS 10 + 16) tend to form four tandem microtubule-binding domain repeat (4R-tau) pathology, while mutations outside exon 10 (i.e., V337M, R406W, Q351R) tend to form mixed 3R/4R tauopathy similar to the tauopathy in Alzheimer's disease (6).

Mutations in the *GRN* linked to chromosome 17q21 initially reported in 2006 (7, 8) could result in a lack of progranulin by haploinsufficiency and the accumulation of TAR DNA-binding protein (TDP)-43 protein (9). *GRN* mutation carriers have a wide range of clinical phenotypes and illness onset ages. The bvFTD, CBS, and PPA are the most common clinical syndromes in patients with *GRN* mutation (10, 11).

The hexanucleotide GGGGCC (G4C2) repeat expansions of the *C9orf72* were identified as a common genetic cause of FTLD and ALS in 2011 (12, 13). TDP-43 aggregations were pathologically discovered in cases with *C9orf72* expansions (14). The most prevalent clinical syndromes were bvFTD, ALS, or a mixture of both in *C9orf72* mutation carriers (14, 15).

Currently, the visual inspection of MRI was demonstrated to easily increase the diagnostic confidence of underlying FTLD (16, 17). The cortical microstructure was found to be more sensitive than cerebral atrophy within patients with GRN mutations (18), suggesting the powerful value of MRI to correctly diagnose and capture the early abnormalities in familial FTLD. Noninvasive quantitative molecular imaging with PET or single-photon emission CT (SPECT) provided another perspective and allowed for selective visualization of the molecular targets *in vivo* to investigate the brain topographic and pathophysiological changes. The former included metabolism, perfusion, neuroinflammation, synaptic function, and neurotransmitters' activity, and the latter comprised Tau and Aβ aggregation. There was increasing evidence that several molecular imaging biomarkers tend to serve as biomarkers to reveal the early brain abnormalities in familial FTLD. Identifying molecular imaging biomarkers for familial FTLD is important for the *in-vivo* assessment of underlying pathophysiological changes with disease progression and future disease-modifying therapy. Thus, we review the recent progress of molecular imaging in familial FTLD with a focus on the possible implication of these techniques and their prospects in specific mutation types.

Methods

Search strategy

We performed electronic searches of Medline, PubMed, and Embase databases using the combination of a number of medical subject headings, Emtree subject headings, and free-text terms ("frontotemporal lobar degeneration," and "frontotemporal dementia" for clinical categories; "microtubule-associated protein tau" or "*MAPT*," "progranulin" or "*GRN*," and "chromosome 9 open reading frame 72" or "*C9orf72*" for genes; "positron emission tomography" or "PET," "single-photon emission CT" or "SPECT," and "dopamine transporter imaging" for molecular imaging biomarkers). The retrieval deadline was 1 December 2021. All the relevant articles were retrieved, placing restrictions on #elds (free-text terms searched exclusively in the title or abstract of the articles) and publication type (original articles).

Discussion

Pathophysiological biomarkers

Tau studies

Tau-PET is currently being explored as a promising method to identify the tau protein *in vivo* (19). Several types of tracers have been applied to map the pattern of tau accumulation in familial FTLD, especially in individuals with *MAPT* mutations thought to be tauopathy. ^{18}F-flortaucipir, the most commonly used tau tracer, has been proven to bind paired helical filaments composed of 3R/4R tau in Alzheimer's disease (AD) (20, 21). In recent years, other tracers, including ^{11}C-PBB3 (22), ^{18}F-MK6240 (23), and ^{18}F-PM-PBB3 (24), started to be applied in *MAPT* mutation carriers. ^{11}C-PBB3 could capture wide-ranging Tau pathologies, including 3R/4R tau and 4R tau (25, 26) compared to^{18}F-flortaucipir (27). For ^{18}F-MK6240 and

TABLE 1 Studies investigating *MAPT* mutation vs. controls.

No.	References	No. of subjects	Techniques	Findings
1	Arvanitakis et al. (43)	(2 a*MAPT*+, 5 s*MAPT*+) vs. 3 NC	^{18}F-FDG PET	Asymmetric temporal lobe hypometabolism in 7 *MAPT*+
2	Laws et al. (44)	(31 s*MAPT*_H1+ 10s *MAPT*_H2) vs. 16 HC	^{18}F-FDG PET	More pronounced hypometabolism in frontal brain areas of H2 carriers than H1 carriers
3	Deters et al. (45)	(3 a*MAPT*+, 8 s*MAPT*+) vs. 8 NC	^{18}F-FDG PET	Hypometabolism bilaterally in the medial temporal lobe, and the parietal and frontal cortices
4	Yang et al. (46)	2 s*MAPT*+ vs. 1 NC	^{18}F-FDG PET	Hypometabolism in extensive prefrontal areas, and hypermetabolism in the putamen, globus pallidum, cerebellum, and sensorimotor cortex
5	Su et al. (24)	1 s*MAPT*+ vs. HC	^{18}F-FDG PET	Brain metabolism significantly decreased in bilateral temporal lobes and moderately decreased in bilateral frontal lobes with more remarkable in the left side
6	Clarke et al. (47)	6 a*MAPT*+ vs. 12 NC	^{18}F-FDG PET	Hypometabolism in the anterior cingulate
7	Bevan Jones et al. (36)	1 s*MAPT*+ vs. 12 HC	^{18}F-flortaucipir PET	Increased tau accumulation in the anterior temporal lobes and ventral anterior cingulate cortex
8	Smith et al. (39)	3 s*MAPT*+ vs. 4 HC	^{18}F-flortaucipir PET	Increased tau accumulation mainly in the hippocampus and adjacent temporal lobe regions of 2 s*MAPT*+ with short disease duration and isolated memory impairment; the temporal, frontal lobes, and basal ganglia of 1 s*MAPT*+ with long disease duration and behavioral deficits
9	Spina et al. (41)	1 s*MAPT*+ vs. 20 HC	^{18}F-flortaucipir PET	Increased tau accumulation in the bilateral frontal pole, medial orbitofrontal cortex, inferior temporal lobe, insular cortex, anterior cingulate, dorsolateral prefrontal cortex, and lateral temporal cortex
10	Jones et al. (34)	(3 a*MAPT*+, 10 s*MAPT*+) vs. 241 HC vs. 30 AD	^{18}F-flortaucipir PET	The greatest tau accumulation in AD and minimal regional tau accumulation in *MAPT*+ with mutations in exon 10
11	Bevan Jones et al. (35)	1 a*MAPT*+ vs. 13 HC	^{18}F-flortaucipir PET	A lack of tau aggregation in frontotemporal regions
12	Tsai et al. (42)	6 s*MAPT*+ vs. 53 HC	^{18}F-flortaucipir PET	Tau depositions in left insula and bilateral temporal poles
13	Convery et al. (37)	1 s*MAPT*+ vs. 6 HC	^{18}F-flortaucipir PET	Baseline: tau aggregation in the insula region cortically, and the medial temporal, putamen, and pallidum regions subcortically Follow-up: tau aggregation in the same regions as at baseline but also the temporal region cortically and caudate and thalamus regions subcortically
14	Soleimani-Meigooni et al. (40)	2 s*MAPT*+ vs. 14 HC	^{18}F-flortaucipir PET	Tau depositions in the temporal lobes, temporal white matter, and basal ganglia
15	Malpetti et al. (48)	2 s*MAPT*+ vs. 15 HC	^{18}F-flortaucipir PET	Consistent tau deposition distribution in frontotemporal regions in 2 s*MAPT*+
16	Ikeda et al. (22)	4 s*MAPT*+ vs. 13 HC	^{11}C-PBB3 PET	Mild tau depositions in the midbrain and medial temporal areas of 2 s*MAPT*+ from kindred with slow progression; profoundly increased tau depositions in widespread regions of 2 s*MAPT*+ from kindreds with rapid progression
17	Su et al. (24)	1 s*MAPT*+ vs. HC	^{18}F-PM-PBB3 PET	Slightly diffuse tau deposition especially in the left frontal lobe
18	Levy et al. (23)	(3 a*MAPT*+, 3 s*MAPT*+) vs. 83 HC	^{18}F-MK-6240 PET	At least mild but significant tau deposition in 3 s*MAPT*+; modest tau deposition in 2 a*MAPT*+ within 5 years from estimated onset; no tau deposition in 1 a*MAPT*+ about 30 years from estimated onset
19	Miyoshi et al. (49)	3 aMAPT+ vs. 9 HC	^{11}C-DOPA PET	Low dopamine synthesis in putamen
20	Yang et al. (46)	2 s*MAPT*+ vs. 1 NC	^{11}C-CFT PET	Dopaminergic dysfunction in the caudate nucleus and putamen

(Continued)

TABLE 1 Continued

No.	References	No. of subjects	Techniques	Findings
21	Wu et al. (50)	(3 a*MAPT*+, 1 s*MAPT*+) vs. 6 HC	^{11}C-CFT PET	Dopaminergic dysfunction is severe in s*MAPT*+ and mild in a*MAPT*+
22	Smith et al. (39)	3 s*MAPT*+ vs. 4 HC	Amyloid-PET (^{18}F-flutemetamol)	Negative in all participants
23	Tsai et al. (42)	5 s*MAPT*+ vs. 53 HC	Amyloid-PET (^{11}C-PiB)	Positive in 1 s*MAPT*+
24	Soleimani-Meigooni et al. (40)	2 s*MAPT*+ vs. 14 HC	Amyloid-PET (^{11}C-PiB)	Positive in 1 s*MAPT*+
25	Su et al. (24)	1 s*MAPT*+ vs. HC	Amyloid-PET (^{18}F-florbetapir)	Negative in 1 s*MAPT*+
26	Levy et al. (23)	(3 a*MAPT*+, 3 s*MAPT*+) vs. 83 HC	Amyloid-PET (^{18}F-flutafuranol)	Negative in all participants
27	Seelaar et al. (51)	10 s*MAPT*+ vs. 10 HC	^{99m}Tc-HMPAO SPECT	Hypoperfusion in the left temporal and inferior frontal gyri
28	Chaunu et al. (52)	1 s*MAPT*+	^{99m}Tc-HMPAO SPECT	Hypoperfusion in the bilateral left predominant frontotemporal and basal ganglia
29	Miyoshi et al. (49)	3 a*MAPT*+ vs. 9 HC	^{11}C-DAA1106 PET	Increased Glial activities in the frontal cortex of 1 a*MAPT*+, the occipital cortex of 2 a*MAPT*+, and the posterior cingulate cortex of 1 a*MAPT*+
30	Bevan-Jones et al. (35)	1 a*MAPT*+ vs. 15 HC	^{11}C-PK11195 PET	Microglial activation in frontotemporal regions
31	Malpetti et al. (48)	2 s*MAPT*+ vs. 15 HC	^{11}C-PK11195 PET	Tau deposition overlapped with that of microglial activation but more extensive
32	Miyoshi et al. (49)	3 a*MAPT*+ vs. 9 HC	^{11}C-MP4A PET	Decreased AChE activity in the temporal, parietal cortex

aMAPT+, asymptomatic MAPT mutation carriers; sMAPT+, symptomatic MAPT mutation carriers; HC, healthy controls; NC, non-carriers; FDG, fluorodeoxyglucose; HMPAO, hexamethylpropylene amine oxime; PiB, Pittsburgh compound B; PET, positron emission tomography; SPECT, single photon emission computed tomography; AChE, acetylcholinesterase.

^{18}F-PM-PBB3, no clear off-target binding was reported with the improved design (28–31). ^{18}F-PM-PBB3 has shown higher binding affinities to 4R tau compared with the 3R/4R tracer ^{18}F-MK6240 (24). The novel tau tracers might help show diverse tau pathologies in various mutation subtypes.

*MAPT*_Tau-PET

Frontotemporal lobar degeneration with *MAPT* mutations is regarded as tauopathy (32), and tau PET provides an effective way to explore biomarkers for multiform tau pathologies in a homogeneous patient group (33). Most individuals with *MAPT* mutations inside exon 10 (i.e., P301L, S305N, N279K) and intron 10 (i.e., IVS 10 + 16) had 4R tau pathology, while with *MAPT* mutations outside exon 10 (i.e., V337M, R406W, Q351R) had 3R/4R paired helical filament tau pathology (34). *MAPT* mutations have different types of underlying tauopathies, leading to different tracer binding patterns.

^{18}F-flortaucipir was most commonly used to track 3R/4R tau, so most participants with *MAPT* mutations outside exon 10 showed a higher-level tracer binding than mutations in exon 10 (34). Only a few ^{18}F-flortaucipir studies included 4 asymptomatic *MAPT* mutation carriers. 3 of them (1 N279K, 1 R406W, and 1 IVS 10 + 16) had little to no uptake, but the other *MAPT* R406W mutation carrier had a signal in the AD range (34, 35). The heterogeneous results might be explained by quite a limited sample size. In symptomatic *MAPT* mutation carriers, temporal (36–42), insular (37, 41, 42), and frontal (38, 39, 41) regions were most commonly reported for increased ^{18}F-flortaucipir uptake. Especially, in two *MAPT* R406W mutation carriers with short disease duration, the hippocampus and adjacent temporal lobe regions were mainly involved, whereas in another *MAPT* R406W mutation carrier with a long duration, tau aggregation spreads across the whole temporal, frontal lobes, and the basal ganglia (39). Moreover, a longitudinal study of a *MAPT* Q351R mutation carrier also demonstrated that tau aggregation expanded from the insula region cortically, and the medial temporal, putamen, and pallidum regions subcortically to the temporal region cortically and caudate and thalamus regions subcortically even just over 1 year (37). These findings suggested that ^{18}F-flortaucipir might be a sensitive biomarker for disease progression in symptomatic *MAPT* mutation carriers. However, the majority of research was based on case reports or cross-sectional studies with small sample size. Longitudinal data with larger cohorts will be required for such investigations.

Two studies applied ^{11}C/^{18}F-PBB3 tracking both the 3R/4R tau and 4R tau in symptomatic *MAPT* mutation carriers (22, 24). In four patients with *MAPT* N279K mutation, the kindred with slow progression exhibited mild binding; in contrast, kindreds with rapid progression showed profoundly increased binding in widespread regions from an early disease stage (22). Recently, a study of ^{18}F-MK-6240 in two asymptomatic *MAPT* P301L mutation carriers showed modest tau deposition about 5 years from estimated onset (23), indicating that ^{18}F-MK-6240 uptake might be an early biomarker for *MAPT* P301L mutation carriers (Table 1).

TABLE 2 Studies investigated asymptomatic/symptomatic *GRN* carriers.

No.	References	No. of subjects	Techniques	Findings
1	Huey et al. (54)	2 s*GRN*+	^{18}F-FDG PET	Predominant right-sided hypometabolism
2	Jacova et al. (55)	9 *GRN*+ (4 a*GRN*+) vs. 11 NC (8 aNC)	^{18}F-FDG PET	*GRN*+ showed an overall pattern of right anterior cerebral hypometabolism
3	Josephs et al. (56)	3 s*GRN*+ vs. 3 sNC vs. 26 HC	^{18}F-FDG PET	s*GRN*+ and sNC vs. HC: left temporoparietal hypometabolism s*GRN*+ vs. sNC: more severe anteromedial temporal hypometabolism
4	Caroppo et al. (57)	Baseline: 16 a*GRN*+ VS 17 NC Follow-up: 14 a*GRN*+ VS 14 NC	^{18}F-FDG PET	Baseline: left middle temporal gyrus hypometabolism Follow-up: left inferior temporal, left middle frontal, left inferior orbital frontal, right superior orbital frontal gyri, left thalamus hypometabolism
5	Licata et al. (58)	10 s*GRN*+ vs. 23 HC	^{18}F-FDG PET	Inter-individual variability of FDG uptake pattern in s*GRN*+. All s*GRN*+ showed frontal hypometabolism. Asymmetrical metabolism in half of s*GRN*+
6	Deng et al. (59)	1 s*GRN*+	^{18}F-FDG PET	Bifrontal and bitemporal hypometabolism
7	Ljubenkov et al. (60)	26 *GRN*+ (18 s*GRN*+) vs. 52 HC	^{18}F-FDG PET	Left-predominant hypometabolism in dorsal prefrontal, anterior cingulate, orbitofrontal, inferior frontal gyrus, insular, lateral parietal, lateral temporal, posterior cingulate, caudate, and thalamic regions Bifrontal hypometabolism was associated with worse clinical symptoms rating
8	Lagarde et al. (53)	1 s*GRN*+ vs. 8 sporadic FTLD	^{18}F-flortaucipir PET	No tau binding in s*GRN*+; tau binding in 5/8 sNC
9	Carecchio et al. (61)	1 s*GRN*+	DaTScan (^{123}I-ioflupane SPECT)	Reduced tracer uptake in the left putamen
10	Deng et al. (59)	1 s*GRN*+	^{18}F-DOPA PET	^{18}F-DOPA: reduced DOPA metabolism in bilateral corpus striatum
11	Josephs et al. (56)	3 s*GRN*+ vs. 3 sporadic FTLD vs. 26 HC	Amyloid-PET (^{11}C-PiB)	Negative in all participants (cut-off score of <1.5). s*GRN*+ had lower PiB-PET ratios compared to sNC
12	Dopper et al. (62)	1 s*GRN*+	^{99m}Tc-HMPAO SPECT	Symmetrical frontoparietal hypoperfusion.
13	Premi et al. (63)	13 s*GRN*+ vs. 13 sporadic FTLD vs. 13 HC	^{99m}Tc-ECD SPECT	s*GRN*+ and sNC vs. HC: hypoperfusion in frontotemporal areas s*GRN*+ vs. sNC: hypoperfusion in anterior cingulate cortex and left dorsolateral prefrontal cortex
14	Carecchio et al. (61)	1 s*GRN*+	perfusion SPECT	Left predominant bifrontal with homolateral parieto-temporal hypoperfusion

GRN+, GRN mutation carriers; NC, non-carriers; HC, healthy controls; GRN+, symptomatic GRN mutation carriers; aGRN+, asymptomatic GRN mutation carriers; FDG, fluorodeoxyglucose; ECD, ethylcysteinate dimer; HMPAO, hexamethylpropylene amine oxime; PiB, Pittsburgh compound B; DaTscan, dopamine transporter scan; PET, positron emission tomography; SPECT, single photon emission computed tomography.

In *MAPT* mutation carriers, the value of tau PET for capturing tau accumulation has been primarily proved, and the tau aggregation patterns were associated with the subtypes of mutations and tracers. Therefore, novel tracers for multiform tau pathologies need to be further explored in longitudinal studies with larger cohorts.

GRN/*C9*orf72_Tau-PET

Three studies reported ^{18}F-flortaucipir binding in the frontotemporal region in five symptomatic *GRN* mutation carriers (38, 40, 42), whereas another research found no ^{18}F-flortaucipir binding in a patient with *GRN* mutation (53) (Table 2). Similarly, findings among symptomatic *C9orf72* mutation carriers were contradictory. Ten patients with *C9orf72* mutation had increased ^{18}F-flortaucipir binding in the frontal lobe (38, 40, 42, 64), while another study found no tau deposition in six patients with *C9orf72* mutation (65) (Table 3). The contradictory results might be associated with the small number of participants and different clinical phenotypes. Moreover, a study showed that ^{18}F-MK-6240 PET scan was negative for three individuals with *GRN* or *C9orf72* mutations (23), implying that ^{18}F-MK-6240 might not be an optimal method for tracking tau deposition in *GRN* or *C9orf72* mutation carriers.

TABLE 3 Studies investigated asymptomatic/symptomatic *C9* carriers.

No.	References	No. of subjects	Techniques	Findings
1	Gramaglia et al. (66)	1 s*C9+*	^{18}F-FDG PET	Bilateral frontotemporal hypometabolism
2	Martikainen et al. (67)	1 s*C9+*	^{18}F-FDG PET	Hypometabolism in temporal lobes
3	Solje et al. (68)	36 s*C9+*	^{18}F-FDG PET	Normal in 17.6% of s*C9+*
4	Block et al. (69)	1 s*C9+*	^{18}F-FDG PET	Symmetric and mild medial-greater-than-lateral bifrontal hypometabolism
5	Sha et al. (70)	1 s*C9+*	^{18}F-FDG PET	Bilateral frontal and temporoparietal hypometabolism
6	Castelnovo et al. (71)	9 s*C9+*	^{18}F-FDG PET	Prevalent frontal hypometabolism in bvFTD *C9+*
7	Diehl-Schmid et al. (72)	22 s*C9+* vs. 22 sporadic FTLD vs. 23 HC	^{18}F-FDG PET	Right temporal polar and lateral hypometabolism in svPPA *C9+* s*C9+* vs. sNC: a significant reduction of glucose metabolism in both thalami
8	Levy et al. (73)	1 s*C9+*	^{18}F-FDG PET	Bifrontal hypermetabolism; no significant areas of hypometabolism
9	Sellami et al. (74)	1 s*C9+*	^{18}F-FDG PET	Bilateral frontal and anterior temporal hypometabolism
10	De Vocht et al. (75)	17 a*C9+* vs. 25 HC	^{18}F-FDG PET	Hypometabolism in frontotemporal regions, basal ganglia, and thalami of a*C9+*
11	Filikci et al. (76)	1 s*C9+*	^{18}F-FDG PET	Hypometabolism in parietotemporal cortex, posterior cingulate gyrus and precuneus, mesial temporal lobes, and frontal lobes
12	Popuri et al. (77)	15 a*C9+* vs. 20 NC	^{18}F-FDG PET	Cingulate gyrus, frontal, and temporal neocortices (left >right) and bilateral thalami hypometabolism
13	Bevan-Jones et al. (64)	1 s*C9+* vs. 13 NC	^{18}F-flortaucipir PET	Increased binding in frontotemporal cortex of sym *C9+*
14	Smith et al. (65)	6 s*C9+* vs. 6 sv PPA vs. 54 HC	^{18}F-flortaucipir PET	*C9+* exhibited none or limited ^{18}F-flortaucipir retention
15	Filikci et al. (76)	1 s*C9+*	DaTScan	Unremarkable DaTscan
16	Martikainen et al. (67)	1 s*C9+*	Amyloid-PET (^{11}C-PiB)	Negative amyloid PET
17	Block et al. (69)	1 s*C9+*	Amyloid-PET	Negative amyloid PET.
18	Sha et al. (70)	1 s*C9+*	Amyloid-PET (^{11}C-PiB)	Positive amyloid PET.
19	Filikci et al. (76)	1 s*C9+*	Amyloid PET (^{11}C-PiB)	Negative amyloid PET
20	Malpetti et al. (48)	3 a*C9+* vs. 1 sporadic FTLD vs. 19 HC	^{11}C-UCB-J PET	a*C9+* vs. HC: reduced synaptic density in the thalamus

C9+, C9orf72 mutation carriers; NC, non-carriers; HC, healthy controls; sC9+, symptomatic C9orf72 mutation carriers; aC9+, asymptomatic C9orf72 mutation carriers; bvFTD, behavioral variant frontotemporal dementia; svPPA, semantic variant primary progressive aphasia; FDG, fluorodeoxyglucose; PiB, Pittsburgh compound B; DaTscan, dopamine transporter scan; PET, positron emission tomography; SPECT, single photon emission computed tomography.

Amyloid studies

To detect the underlying AD pathology, amyloid-PET with tracers, including ^{11}C-Pittsburgh compound B (PiB) (42, 67, 70, 76), ^{18}F-florbetapir (24), ^{18}F-florbetaben (23), ^{18}F-flutafuranol (78), ^{18}F-flutemetamol (39, 79), etc., is applied in patients with familial FTLD.

Most patients with *MAPT* mutation indicated negative results with ^{11}C-PiB or ^{18}F-florbetapir PET (23, 24, 39, 42), while two patients with *MAPT* P301L mutation had a positive ^{11}C-PiB scan (40, 42). However, one might imply an incidental rather than preclinical β-amyloid pathology since the SUVRs were well below those seen in AD (42); in contrast, the other regarded as combining with AD presented higher SUVRs close to AD (40). Negative results with ^{11}C-PiB or ^{18}F-flutafuranol were reported in patients with *GRN* and *C9orf72* mutation carriers so far (23, 42, 56, 76). Thus, amyloid-PET may help discriminate true underlying AD co-pathology from incidental β-amyloid pathology (80) (Table 4).

Topographic biomarkers

Brain metabolism

^{18}F-fluorodeoxyglucose (FDG)-PET is a technique for measuring glucose metabolism *in vivo* (82). Studies of FDG-PET could capture the different patterns of brain hypometabolism and even precede brain atrophy in familial FTLD mutation carriers (43, 45, 47, 55, 57, 72, 83).

*MAPT*_FDG-PET

A few cross-sectional FDG-PET studies demonstrated brain hypometabolism in both the asymptomatic and symptomatic

TABLE 4 Studies investigating multiple different mutations in FTLD.

No.	References	No. of subjects	Techniques	Findings
1	Tsai et al. (42)	6 s*MAPT*+ vs. 5 s*C9*+ vs. 1 s*GRN*+ vs. 53 HC	^{18}F-flortaucipir PET	Tau deposition in the left insula and bilateral temporal poles of s*MAPT*+; the left lateral frontal, parietal and temporal lobes of s*GRN*+; the frontal poles of s*C9*+ with varying degrees
2	Soleimani-Meigooni et al. (40)	2 s*MAPT*+ vs. 1 s*C9*+ vs. 1 s*GRN*+ vs. 14 HC	^{18}F-flortaucipir PET	Tau deposition was less than Alzheimer's disease, though higher than HC, and did not reliably correspond with post-mortem tau pathology for all mutation groups
3	Malpetti et al. (48)	2 s*MAPT*+ vs. 3 s*C9*+ vs. 2 s*GRN*+ vs. 15 HC	^{18}F-flortaucipir PET	Consistent tau deposition distribution (overlapped with that of ^{11}C-PK11195, but was more extensive) in 2 s*MAPT*+, heterogeneous tau deposition distributions among s*GRN*+ and s*C9*+
4	Levy et al. (23)	(3 a*MAPT*+, 3 s*MAPT*+) vs. 2 s*C9*+ vs. 2 s*GRN*+ vs. 83 HC	^{18}F-MK-6240 PET	At least mild but significant tau deposition in 3 s*MAPT*+; modest tau deposition in 2 a*MAPT*+ within 5 years from estimated onset; no tau deposition in 1 a*MAPT*+ about 30 years from estimated onset Negative for 2 s*GRN*+, and 1 advanced s*C9*+ showed minimal regionally non-specific binding
5	Tsai et al. (42)	5 s*MAPT*+ vs. 4 s*C9*+ vs. 1 s*GRN*+ vs. 53 HC	Amyloid-PET (^{11}C-PiB)	Positive in 1 s*MAPT*+ and 1 s*GRN*+
6	Levy et al. (23)	(3 a*MAPT*+, 3 s*MAPT*+) vs. 2 s*C9*+ vs. 2 s*GRN*+ vs. 83 HC	Amyloid-PET (^{18}F-flutafuranol)	Negative in all participants
7	Seelaar et al. (51)	10 s*MAPT*+ vs. 19 FTLD-TDP (6 *GRN*+, 5 Ser82ValfsX174+, 1 Gln125X+, 13 unknown gene defect) vs. 10 HC	^{99m}Tc-HMPAO SPECT	Hypoperfusion in the right frontal lobe, precuneus, cuneus, and inferior parietal lobule of familial FTLD-TDP; in the left temporal and inferior frontal gyri of *MAPT*+
8	Lant et al. (81)	10 s*MAPT*+ vs. 9 s*C9*+ vs. 8 s*GRN*+ vs. 13 AD vs. 13 HC	^{11}C-PK11195 PET	Significantly microglial activation in all four regions (cortical gray and subcortical white matter of frontal and temporal) of FTLD Greater microglial activation of frontal subcortical white matter in FTLD than AD, temporal cortical gray matter in contrast Microglial activation was higher in FTLD-*MAPT* than other genetic forms (*GRN*, *C9*)
9	Malpetti et al. (48)	2 s*MAPT*+ vs. 3 s*C9*+ vs. 2 s*GRN*+ vs. 15 HC	^{11}C-PK11195 PET	Increased microglial activation predominantly in frontotemporal regions for all mutation groups

FTLD, frontotemporal lobar degeneration; TDP, TAR DNA binding protein; aMAPT+, asymptomatic MAPT mutation carriers; sMAPT+, symptomatic MAPT mutation carriers; sC9+, symptomatic C9orf72 mutation carriers; sGRN+, symptomatic GRN mutation carriers; HC, healthy controls; NC, non-carriers; HMPAO, hexamethylpropylene amine oxime; PiB, Pittsburgh compound B; PET, positron emission tomography; SPECT, single photon emission computed tomography.

MAPT mutation carriers (24, 43, 45–47). Hypometabolism in the temporal lobe (43, 45) and anterior cingulate cortex (47) was reported in asymptomatic *MAPT* mutation carriers, while temporal lobe hypometabolism even preceded the brain atrophy on MRI in the asymptomatic stage (43). In symptomatic *MAPT* mutation carriers, hypometabolism regions spread extensively to the frontotemporal lobes (24, 43, 46), while hypermetabolism was also found in the putamen, globus pallidum, cerebellum, and sensorimotor cortex (46). These findings all pointed to early involvement of the temporal lobe in asymptomatic *MAPT* mutation carriers. Furthermore, only one study compared three asymptomatic *MAPT* mutation carriers and 8 symptomatic

MAPT mutation carriers, but found no difference in FDG uptake (45), which was mainly due to the small sample size. However, most current studies were cross-sectional with a small cohort, and further studies are needed to characterize the trajectories of metabolism patterns from asymptomatic to symptomatic *MAPT* mutation carriers.

*GRN*_FDG-PET

Two studies indicated asymmetric temporal lobe hypometabolism with FDG-PET in asymptomatic *GRN* mutation carriers (55, 57). After 20 months of follow-up, hypometabolism spread to the frontal lobe and thalamus (57). The metabolic changes appeared before brain atrophy on MRI and approximately more than 10 years before clinical onset (57), suggesting that FDG-PET changes can be detected as early biomarkers in *GRN* mutation carriers. In symptomatic *GRN* mutation carriers, the asymmetrical hypometabolism of temporoparietal (56) and frontal (58) lobes was reported primarily based on a small number of cross-sectional studies or case reports. Hypometabolism patterns were observed to correlate with clinical manifestations (56), but another study failed to find clear metabolic change pattern in each clinical subtype (58).

*C9orf72*_FDG-PET

In asymptomatic *C9orf72* mutation carriers, extensive hypometabolism was observed in frontotemporal and subcortical regions in two studies (75, 77). Thalami hypometabolism was found in both the asymptomatic (75, 77) and symptomatic (72) individuals with *C9orf72* mutation, especially when compared to sporadic FTLD patients (72), suggesting that thalami could be a distinguishing early biomarker for *C9orf72* mutation carriers. In symptomatic *C9orf72* mutation carriers, some studies showed that the hypometabolism patterns were consistent with the clinical diagnosis and correlated well with the brain atrophy on MRI, for example, prevalent frontal hypometabolism in patients with bvFTD and temporal polar and lateral temporal hypometabolism in patient with svPPA (66, 69, 71, 74). However, the cross-sectional studies above with small sample sizes still need to be replicated in longitudinal studies with larger cohorts.

Most studies demonstrated the concordance between structural MRI and FDG-PET in *MAPT* (43, 45), *GRN* (84, 85), and *C9orf72* (74, 77) mutation carriers. However, controversy still existed regarding the earlier or more sensitive biomarkers (43, 45, 77). Some studies showed that additional informative MRI modalities such as diffusion tensor imaging (DTI) and arterial spin labeling (ASL) had equivalent or even better diagnostic utility of FTLD compared with FDG-PET (86–89), but others found a gap in sensitivity or accuracy that still remained (90, 91). Further investigations of familial FTLD need to compare the clinical value of microstructural MRI and PET.

Dopaminergic system

Dopamine functional deficits can be measured *in vivo via* PET or SPECT with various types of tracers assessing dopamine synthesis and storage [^{18}F-DOPA, ^{11}C-DOPA, ^{11}C-dihydrotetrabenazine (DTBZ), ^{18}F-fluoropropyl-DTBZ, etc.], transporter density (^{123}I-FP-CIT, ^{123}I-ioflupane,^{11}C-CFT, ^{99m}Tc-TRODAT, etc.), or postsynaptic terminals [^{11}C-raclopride, ^{123}I-iodobenzamide (IBZM), etc.] (92). Dopaminergic deficits were evaluated by the techniques mentioned above, especially in patients with familial FTLD with Parkinsonism.

Parkinsonism may present as the initial symptom in *MAPT* mutation carriers, particularly individuals with *MAPT* N279K mutation. Tracers such as ^{11}C-DOPA and 2b-carbomethoxy-3b-(4-trmethylstannylphenyl) tropane (^{11}C-CFT) were used to reveal dopaminergic function. The ^{11}C-CFT uptake in the putamen was mildly low in asymptomatic *MAPT* N279K mutation carriers (49, 50). In symptomatic patients, both the caudate nucleus and putamen were involved more heavily (46, 50).

Individuals with *GRN* mutations and Parkinsonism could show reduced DOPA metabolism in bilateral corpus striatum by ^{18}F-DOPA PET (59) or reduced tracer uptake in left putamen by ^{123}I-ioflupane SPECT (61). Parkinsonism is not uncommon in *GRN* mutation carriers and sporadic patients with FTLD.

Brain perfusion

Perfusion SPECT is a well-established technique for measuring regional cerebral blood flow (rCBF) to assess brain function (93). The tracers utilized in brain perfusion SPECT are technetium-99m-hexamethylpropyleneamineoxime (^{99m}Tc-HMPAO) and technetium-99m-ethylcysteinate dimer (^{99m}Tc-ECD), both which are distributed proportionally to rCBF (93). Perfusion imaging has been widely used in the clinical evaluation of patients with neurological and psychiatric diseases (94), including FTLD.

In 11 *MAPT* mutation carriers, including eight in P301L, two in G272V, and one in G389R, significant hypoperfusion detected by ^{99m}Tc-HMPAO SPECT was found in the asymmetric frontotemporal lobes (51, 52). Several studies indicated that hypoperfusion occurred in frontal areas of *GRN* mutation carriers (61–63). Compared with *MAPT* mutation carriers, patients with *GRN* mutation exhibited relatively more posterior hypoperfusion, including the precuneus and inferior parietal lobule detected by ^{99m}Tc-HMPAO SPECT (51). Perfusion SPECT might be a potential biomarker to identify *MAPT* and *GRN* mutation carriers.

Neuroinflammation

Previous studies of genome-wide association (95) and animal (96) suggest that neuroinflammation might be an

earlier process in FTLD, even preceding tau accumulation. The neuroinflammation is accompanied by the activation of microglia, and 18 kDa TSPO, previously known as peripheral benzodiazepine receptors, is highly expressed (97). Thus, radioligands (^{11}C-PK11195, ^{11}C-DAA1106) have been developed to target TSPO to visualize neuroinflammation *in vivo* (98, 99).

In asymptomatic *MAPT* mutation carriers, two studies with ^{11}C-PK11195 PET (35) or ^{11}C-DAA1106 PET (49) revealed increased levels of microglial activation, even despite a lack of significant atrophy or ^{18}F-flortaucipir uptake (35). In symptomatic patients, ^{18}F-flortaucipir binding overlapped with ^{11}C-PK11195 binding and was more extensive across the brain (38). These findings suggest that neuroinflammation might facilitate tau aggregation initially, then tau-mediated neurodegeneration takes the dominant role. Combining different modalities in a relatively homogeneous group such as familial FTLD with a specific mutation subtype would better understand the underlying mechanism of disease progression.

Across different mutation subtypes, familial patients with FTLD with *MAPT*, *GRN*, and *C9orf72* mutations all showed increased ^{11}C-PK11195 binding predominantly in frontotemporal regions (38), and ^{11}C-PK11195 binding was significantly higher in temporal subcortical white matter in *MAPT* mutation carriers than in other genetic (*GRN*, *C9orf72*) mutation carriers or sporadic FTLD (81). Future studies could add more details to the neuroinflammation patterns of subtypes of familial FTLD.

Synaptic function and acetylcholinesterase activity

The synaptic vesicle glycoprotein 2A (SV2A) is a transmembrane protein ubiquitously expressed in secretory vesicles of synapsis in all the brain areas (100). It is critical for synaptic function (101), and it has been related to neurologic disorders such as AD and epilepsy (102–104). The density of SV2A could be quantified by the newly developed tracer ^{11}C-UCB-J (105). Reduced synaptic density in the thalamus detected by ^{11}C-UCB-J was found in three asymptomatic *C9orf72* mutation carriers compared to healthy controls. It proved the role of the thalamus in *C9orf72* mutation carriers again, especially before symptom onset (48). There is a lack of studies on synaptic density mapping in other early staged mutation carriers. Thus, its value and correspondence with other imaging techniques remain unknown.

^{11}C-MP4A PET could reflect acetylcholinesterase (AChE) activity *in vivo*. A study showed reduced AchE activity in the temporoparietal cortex in one of three asymptomatic *MAPT* N279K mutation carriers (49). Therefore, more studies with larger sample sizes are needed to provide further evidence for ^{11}C-MP4A PET in familial FTLD.

Challenges and limitations of molecular imaging

Even though more and more tracers were approved by the US Food and Drug Administration and by the European Medicines Agency for clinical usage (106), the higher cost and longer acquisition times compared to MRI might limit the wide applications in clinical practice (107). Changes in the levels of human fluid components could reflect underlying pathophysiological processes, and several fluid biomarkers were available or showed potential values such as Aβ, tau, NfL, and progranulin. A lack of multicenter standardization of procedures and quality control would compromise the stability and reliability of outcomes (108). By contrast, molecular imaging could provide more robust and comprehensive (quantitative and spatial distribution) information. However, the unspecific binding was still a challenge. Off-target binding of first-generation tau tracers such as ^{18}F-flortaucipir might interfere with the quantification in several brain regions (109). Further development of 4R tau and TDP-43 specific tracers was needed to move toward precise diagnoses in FTLD. Several studies demonstrated that some molecular imaging biomarkers of FTLD with mutations could be different from sporadic individuals (72, 81), suggesting findings in genetic FTLD that may not translate to sporadic FTLD.

Conclusion

This review summarized recent molecular imaging findings in familial frontotemporal lobar degeneration regarding common genetic mutations. The application of advanced neuroimaging techniques in monogenetic familial FTLD provides a unique opportunity to study specific proteinopathies and their clinical phenotypes. Although various study designs and data analysis methods generated heterogeneous nonspecific results, some key biomarkers could still be identified, pointing to specific brain regions worth further exploring. The combination of multimodal neuroimaging would also help identify the underlying mechanism of these biomarkers. To date, this research topic has been limited by a large multicenter longitudinal cohort study and a comparison between asymptomatic/symptomatic mutation carriers and sporadic patients with FTLD. Thus, the changes in different time points of these biomarkers between FTLD mutation carriers and sporadic ones are largely unknown, and the prognostic value of these biomarkers is still unclear. Future

studies could focus on these issues and provide more insight into the significance of these molecular imaging methods and their findings.

Author contributions

RW contributed to data collection, analysis and interpretation of the data, and drafting of the manuscript. HG contributed to analysis and interpretation of the data and drafting of the manuscript. HX and ZJ revised the manuscript. QC contributed to design the study, interpretation of the data, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ANXA11 mutations are associated with amyotrophic lateral sclerosis–frontotemporal dementia

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Background: The Annexin A11 (*ANXA11*) gene has been newly identified as a causative gene of amyotrophic lateral sclerosis (ALS) with or without frontotemporal dementia (FTD). The current study aimed to investigate the *ANXA11* mutations in a Chinese ALS–FTD or FTD cohort.

Methods: We included ten probands/patients with suspected ALS–FTD or FTD. Mutational analysis of *ANXA11* was performed through Next Generation Sequencing (NGS) and Sanger sequencing. We collected and reviewed clinical presentation, neuropsychology test results, brain-imaging findings, and electrophysiological examination findings.

Results: In total, six probands presented with ALS–FTD, and four with behavior variant FTD (bv-FTD). We identified a non-synonymous heterozygous mutation (c.119A>G, p.D40G) of *ANXA11* in proband 1, which is associated with ALS. However, this is the first report of the mutation causing ALS–FTD. Proband 1 started with abnormal behavior and progressed to classic upper motor nervous disease. Magnetic resonance imaging (MRI) showed significant bilateral temporal lobe atrophy and bilateral hyperintensities along the corticospinal tracts.18F-AV45-PET imaging showed negative amyloid deposits.

Conclusion: *ANXA11*-related diseases have high clinical and genetic heterogeneity. Our study confirmed the contribution of *ANXA11* mutations to ALS–FTD. The *ANXA11* mutations established a complex genotype–phenotype correlation in ALS–FTD. Our research further elucidated the genetic mechanism of ALS–FTD and contributed to setting the foundation of future targeted therapy.

KEYWORDS

annexin A11, *ANXA11*, amyotrophic lateral sclerosis, frontotemporal dementia, genotype, phenotype [mesh]

Introduction

Amyotrophic lateral sclerosis, a lethal progressive neurologic disease, is characterized by selective degeneration of the lower and upper motor neurons. Approximately 5–10% of patients with ALS have a positive family history, suggesting that genetic factors substantially contribute to its pathogenesis. Frontotemporal dementia (FTD) is a spectrum of syndromes characterized by a progressive deterioration in behavior, personality, language, and cognition, associated pathologically with frontotemporal lobar degeneration (FTLD). ALS is closely related to FTD. Up to ∼50% of patients with ALS show behavioral dysfunction and/or subtle cognitive impairment, while about 15% meet the psychiatry diagnostic criteria of FTD (termed as ALS–FTD) (1–3). A similar scenario is observed in FTD. Approximately 30% of patients with FTD have motor impairments, and 12.5% meet the diagnostic criteria for ALS (4, 5).

In the past few years, owing to the rapid development of next-generation sequencing, ALS–FTD-associated genes have been progressively identified. For example, mutations of *C9orf72*, *TARDBP*, and *TBK1* have been identified as major genetic causes of ALS–FTD. The aggregation of TAR DNA-binding protein 43 (TDP-43) in the affected brain regions and motor neurons is a common pathological characteristic of each of these variants (6–10) in up to 97% of ALS and 50% of FTD cases. Beyond that, mutations in *CCNF*, *CHCHD10*, *FUS*, *SQSTM1*, *UBQLN2*, and *VCP* are also associated with ALS–FTD (11). However, the genetic etiology of ALS–FTD in some patients remains unclear. In the current study, mutation in the Annexin A11(*AXAN11*) gene was proved to be linked to ALS–FTD in a Chinese clinical cohort. We also included a review of previously reported mutations with ALS or ALS–FTD in the *AXAN11* gene.

Patients and methods

Patients

In total, ten probands/patients with suspected ALS–FTD or FTD from the Department of Neurology, China–Japan Friendship Hospital in Beijing, were enrolled in the study from July 2019 to January 2022. The clinical characteristics, brain imaging results, and laboratory profiles were collected. This research was approved by the institutional board of the Ethics Committees of China–Japan Friendship Hospital in Beijing and followed the Declaration of Helsinki.

Mutation analysis

Genomic DNA was extracted from peripheral blood samples collected from ten suspected patients and healthy volunteers, according to standard procedures. The repeat length of the pathogenic *C9orf72* GGGGCC repeat expansion was examined and excluded in these patients using polymerase chain reaction (PCR) amplification combined with microfluidic capillary electrophoresis.

Whole-exome sequencing was performed following the Illumina specifications. The isolated DNAs were firstly fragmented into 200–250 bp lengths by sonication. Then, DNA libraries were built using the KAPA Library Preparation Kit (Kapa Biosystems, KR0453) and sequenced *via* the Illumina Noveseq s4 platform (Illumina, San Diego, USA) with 150-bp paired-end reads. The human reference genome (UCSC hg19) was applied to the filter and aligned with the raw data using the Burrows-Wheeler Alignment tool (BWA-0.7.12, http://bio-bwa.sourceforge.net/). GATK software (www.broadinstitute.org/gatk) was used to identify single-nucleotide polymorphisms (SNPs), insertions, and deletions (indels). VEP [Ensemble Variant Effect Predictor, McLaren et al. (12)] was used to annotate all the variants, including the genetic position, type, allele frequency, conservation prediction, etc.

Pathogenicity assessment

All the variants were filtered first against the 1,000 genomes project database, for a minor allele frequency (MAF) $\geq$ 1%, and ExAC hom AC $\geq$3. The obtained variants were further selected according to co-segregation, the genetic model, and an MAF $<$ 1% in three databases (1,000 genomes project_EAS, ExAC, and gnomAD_EAS). We then focused on analyzing variants of the ALS-related genes, which were included in the OMIM database. All the candidate pathogenic variants were confirmed by Sanger sequencing and classified according to the American College of Medical Genetics and Genomics (ACMG) standards (13). Finally, the *ANXA11* mutations were selected based on their clinical relevance and pathogenicity.

Electrophysiological studies

For electrophysiological profiles, examinations were conducted using conventional equipment and according to the standard methods, with skin temperatures maintained between 32 and 34°C. Nerve conduction and needle electromyography (EMG) examinations were conducted on 10 patients.

MR technique and protocol

All the patients underwent 3.0T MRI with a device using eight-channel head coils (Discovery MR750 scanner; GE Medical Systems, United States) in the China–Japan Friendship

TABLE 1 Clinical features of ten probands/patients.

	Proband1	Proband2	Proband3	Proband4	Proband5	Proband6	Patient7	Patient8	Patient9	Patient10
Age(y) at onset	66 Y	72 Y	68 Y	51 Y	61 Y	34 Y	70 Y	72 Y	78 Y	80 Y
Disease duration (months)	18 M	36 M	24 M	12 M	8 M	12 M	24 M	12 M	15 M	18 M
Gender (M/F)	F	M	M	M	M	F	M	M	F	F
Education (years)	9	6	9	12	6	16	12	9	6	2
Family history	Limb weakness (1 brother)	Limb weakness (1 brother)	Limb weakness (1 brother)	Limb weakness (1 brother)	Limb weakness (his mother)	Limb weakness (1 sister + her mother)	No	No	No	No
Cognitive sign	Behavioral executive deficits anomia	Behavioral executive deficits anomia	Executive deficits	Executive deficits	Executive deficits anomia	Executive deficits	Behavioral executive deficits anomia	Behavioral executive deficits anomia	Behavioral executive deficits anomia	Behavioral executive deficits anomia
MMSE	25	22	23	26	22	27	23	22	21	19
MOCA	21	19	20	22	20	25	19	20	19	18
DST-Forwards	7	8	7	8	8	8	7	7	7	7
DST-Backwards	5	5	5	6	5	6	5	6	5	5
VFT	20	19	21	49	21	50	45	43	30	21
TMT B-A time (second)	219	244	200	50	231	100	120	110	150	200
RAVLT LOT	30	34	31	39	29	40	42	39	41	40
RAVLT A30 min	10	10	10	11	11	12	10	9	8	9
BNT	20	18	22	25	21	24	23	21	20	20
StroopCWT	30	31	31	40	29	39	30	31	39	34
APOE with e4 allele	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Site of onset	Bulbar + Upper limb	Upper limb	Upper limb	Upper limb	Upper limb	Upper limb + Lower limb	No	No	No	No
ALS clinical features	Dysphagia Dysarthria Limbs weakness Fasciculations Pyramidal signs	Dysarthria Limbs weakness Fasciculations Pyramidal signs	Dysarthria Limbs weakness Fasciculations Pyramidal signs	Limbs weakness Fasciculations Pyramidal signs	Dysarthria Limbs weakness Fasciculations Pyramidal signs	Dysarthria Limbs weakness Fasciculations muscluar atrophy Pyramidal signs	No	No	No	No

(Continued)

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TABLE 1 (Continued)

	Proband1	Proband2	Proband3	Proband4	Proband5	Proband6	Patient7	Patient8	Patient9	Patient10
Needle EMG	Neurogenic lesion in the cervical, thoracic, and lumbosacral spinal cord	Neurogenic lesion in the cervical, thoracic, and lumbosacral spinal cord	Neurogenic lesion in the bulbar, cervical, thoracic, and lumbosacral spinal cord	Neurogenic lesion in the cervical, thoracic, and lumbosacral spinal cord	Neurogenic lesion in the cervical and thoracic spinal cord	Neurogenic lesion in the bulbar, cervical, thoracic, and lumbosacral spinal cord	Normal	Normal	Normal	Normal
Brain MRI	Bilateral temporal lobe atrophy	Bilateral frotal and temporal lobe atrophy	Bilateral temporal lobe atrophy	Left temporal lobe atrophy	Bilateral temporal lobe atrophy	Bilateral temporal lobe atrophy	Bilateral frotal lobe and left temporal lobe atrophy	Bilateral frotal and temporal lobe atrophy	Bilateral frotal lobe and right temporal lobe atrophy	Bilateral frotal and temporal lobe atrophy
18F-AV45-PET	Negative	Negative	N/A	N/A	N/A	N/A	Negative	N/A	Negative	Negative
Diagnosis	bv-FTD +ALS	bv-FTD +ALS	bv-FTD +ALS	bv-FTD +ALS	bv-FTD +ALS	bv-FTD +ALS	bv-FTD	bv-FTD	bv-FTD	bv-FTD
Gene	ANXA11 (c.119A>G,p.D40G)	N	N	N	N	N	N	N	N	N

MMSE, mini-mental state examination scale; MoCA, Montreal cognitive assessment scale; DST, Digit span test; VFT, verbal fluency test; TMT, trail making test; RAVLT, Rey auditory verbal learning test; BNT, Boston word naming test; Stroop CWT, Stroop color word test; ALS–FTD, behavioral variant frontotemporal dementia with amyotrophic lateral sclerosis; bv–FTD, behavioral variant frontotemporal dementia; N/A, not applicable; N, no pathogenic gene mutation was found; 18F-AV45-PET, 18F-florbetapir (AV45) positron emission tomography (PET) imaging.

Hospital. The sequences performed included T1- and T2-weighted fluid-attenuated inversion recovery (FLAIR) and standard coronal T2-weighted sequences.

18F-AV45-PET examination

In total, five patients were selected for 18F-AV45 PET scans using the Discovery Elite scanner (GE Healthcare) at the Tiantan Hospital. 18F-AV45 PET was performed at 20 min and 50 min postinjection of 248 ± 58 MBq. 18F-AV45 PET profiles were analyzed using an ordered subset expectation maximization algorithm with weighted attenuation. Images were smoothed using a 5 mm Gaussian kernel with scatter correction and evaluated prior to the analysis of patient motion and adequacy of statistical counts. Finally, the standardized uptake value ratios (SUVRs) were computed and normalized according to the cerebellar gray matter reference region and the mean activity, from 50 to 70 min.

Literature review

We searched and reviewed published reports of *ANXA11* mutations using PubMed. Clinical, biochemical, neuroimaging, and genetic data from individual references were sourced and compared with the corresponding results of our research.

Result

Clinical features

The current cohort included 10 patients with behavioral variant FTD (bv-FTD). In total, six had probable bv-FTD with ALS according to the Rascovsky criteria. The clinical characteristics of the current Chinese clinical cohort are displayed in Table 1.

All 10 patients (6 men and 4 women) diagnosed with bv-FTD were from the Chinese mainland. The onset of symptoms occurred at the age of 34–80 years, median (IQR) is 69 (58.5–73.5). All 10 patients showed behavioral and executive deficits, and anomia. There were six patients with positive family histories. In total, one proband initially presented with dysarthria, and five probands presented with limb weakness as the initial symptom. Initially, Proband 1 presented with euphoria, loss of manners, impulsiveness, rash behavior, and difficulty cooking at the age of 66 years. A few months later, her speech became slurred, and she had difficulties in expressing and naming. The patient also had gradual weakness in both upper limbs. Fasciculations, hyperreflexia, and positive Babinski sign of the limbs were observed. EMG demonstrated a neurogenic lesion in the cervical, thoracic,

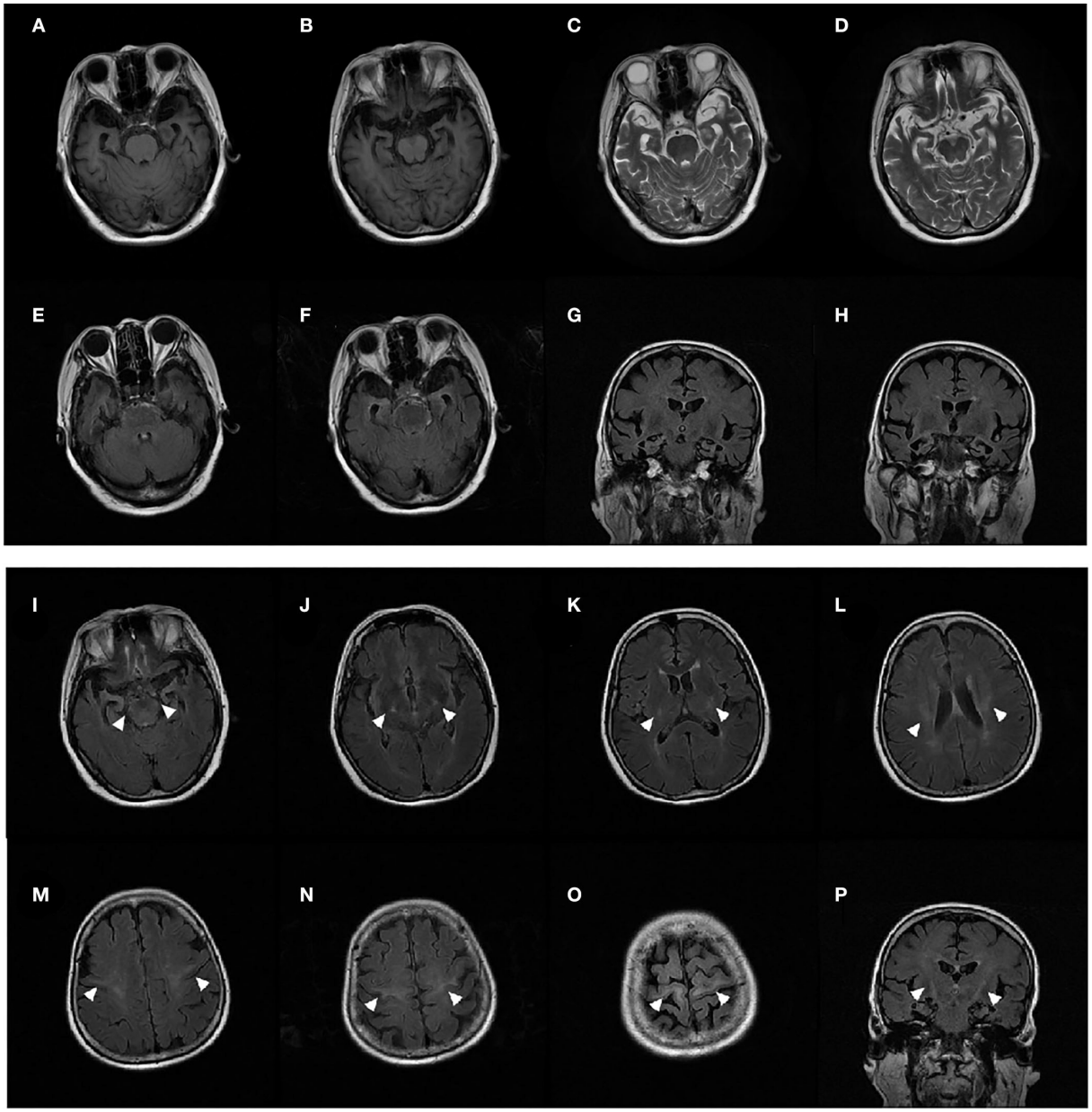

FIGURE 1
MRI of patient 1 showed significant bilateral temporal lobe atrophy **(A–H)**. T2-weighted fluid-attenuated inversion recovery (FLAIR) coronal and axial MRI displayed bilateral signal hyperintensities along the corticospinal tracts in the primary motor cortex, centrum semiovale, posterior limb of the internal capsule, and the cerebral peduncle **(I–P)** (arrows).

and lumbosacral spinal cord. Brain MRI showed bilateral temporal lobe atrophy and bilateral signal hyperintensities along the corticospinal tracts (Figure 1). 18F-AV45-PET imaging showed negative amyloid deposits. The patient was diagnosed as having ALS with bv-FTD. She had an older brother who developed limb atrophy and weakness at 55 years of age and died at 67 years without providing a peripheral blood sample.

ANXA11 mutations and the updated genotype–phenotype spectrum

We identified one non-synonymous heterozygous mutation (c.119A>G, p.D40G) in *ANXA11*, which was previously reported to be associated with ALS, but to our knowledge, this is the first time that has been found in ALS–FTD. By reviewing previous literature

TABLE 2 Clinical and genetic characteristics of *ANXA11*-related diseases.

Gene	Ethnicity	Nucleotide changes	Amino acid changes	Variants type/Zygo	Clinic features	References
ANAX11	British	103C > G	Pro35Ala (P35A)	Missense (Het)	ALS	(14)
	Chinese, Korean	107C > G	Pro36Arg (P36R)	Missense (Het)	ALS, ALS-FTD	(15, 16)
	Euramerican, Korean, South African	112G > A	Gly38Arg (G38R)	Missense (Het)	ALS, ALS-FTD	(16–20)
	French, Brazilian	118G > T	Asp40Tyr (D40Y)	Missense (Het)	ALS, ALS-FTD, hIBM	(19, 21, 22)
	European, Chinese, Korean	119A > G	Asp40Gly (D40G)	Missense (Het)	ALS, ALS-FTD	(15–17), This study
	German	137C > T	Ala46Val (A46V)	Missense (Het)	ALS	(18)
	Chinese	174-2A > G	A58_Q187del	Canonical-Splice (Het)	ALS	(15)
	German	259C > A	Pro87Thr (P87T)	Missense (Het)	ALS	(18)
	Chinese	382G > A	Val128Met (V128M)	Missense (Het)	ALS	(15)
	Korean	409G > A	Gly137Arg (G137R)	Missense (Het)	ALS	(16)
	German	484G > A	Gly162Arg (G162R)	Missense (Het)	ALS	(18)
	British	523G > A	Gly175Arg (G175R)	Missense (Het)	ALS	(17)
	British	566G > A	Gly189Glu (G189E)	Missense (Het)	ALS	(17)
	French	629G > A	Arg210Gln (R210Q)	Missense (Het)	ALS	(19)
	Chinese	687T > A	Ser229Arg (S229R)	Missense (Het)	ALS	(15)
	Korean	c.682_690del9ins TTGTTGT	G228Lfs*29	Frameshift (Het)	ALS	(16)
	British	704G > A	Arg235Gln (R235Q)	Missense (Het)	ALS	(17)
	French	760C > G	Leu254Val (L254V)	Missense (Het)	ALS	(19)
	Spanish	832A > G	Ile278Val (I278V)	Missense (Het)	ALS-FTD	(23)
	Chinese	878C > T	Ala293Val (A293V)	Missense (Het)	ALS	(24)
	Chinese	904C > T	Arg302Cys (R302C)	Missense (Het)	ALS	(15)
	Chinese	921C > G	Ile307Met (I307M)	Missense (Het)	ALS	(24)
	Korean	962C > A	Thr321Asn (T321N)	Missense (Het)	ALS	(16)
	British	1036C > T	Arg346Cys (R346C)	Missense (Het)	ALS	(17)
	Taiwanese	1085A > T	Gln362Leu (Q362L)	Missense (Het)	ALS	(25)
	Japanese	1086 + 1G > A		Canonical-Splice (Het)	ALS	(26)
	German	1087–1G > A		Canonical-Splice (Het)	ALS	(18)
	Korean	1169A > C	His390Pro	Missense (Het)	ALS	(16)
	Chinese	1146_1175del	L383_V392del	Gross deletion (Het)	ALS	(15)
	Korean	1367G > A	Arg456His (R456H)	Missense (Het)	ALS	(16)
	Korean	1458 + 7G > A	I472Sfs*8	Splice (Het)	ALS	(16)
	Chinese	1471G > A	Gly491Arg (G491R)	Missense (Het)	ALS-FTD	(15)

ALS, amyotrophic lateral sclerosis; ALS–FTD, amyotrophic lateral sclerosis-frontotemporal dementia; hIBM, inclusion body myopathy; Het, heterozygous mutation.

in the Human Gene Mutation Database (HGMD), we found out thirty-two different *ANXA11* variants have been identified in ALS and/or ALS–FTD, including patients from the United Kingdom, Southern Africans, Brazil, France, German, Korea, Spain, Japan, and China (Table 2) (14–26). To further investigate the correlation between phenotype and genotype, we reviewed and summarized all the studies on *ANXA11* mutations (Figure 2).

Discussion

Located on the human chromosome 10q22.3, the *ANXA11* gene encodes the 505 amino acid annexin A11 protein, which is a member of a calcium-dependent phospholipid-binding annexin protein family. The primary function of the annexin protein family is to bind Ca2+, RNA, other proteins, and lipid membranes. Unlike other family members, *ANXA11* shows a uniquely long N-terminal domain that

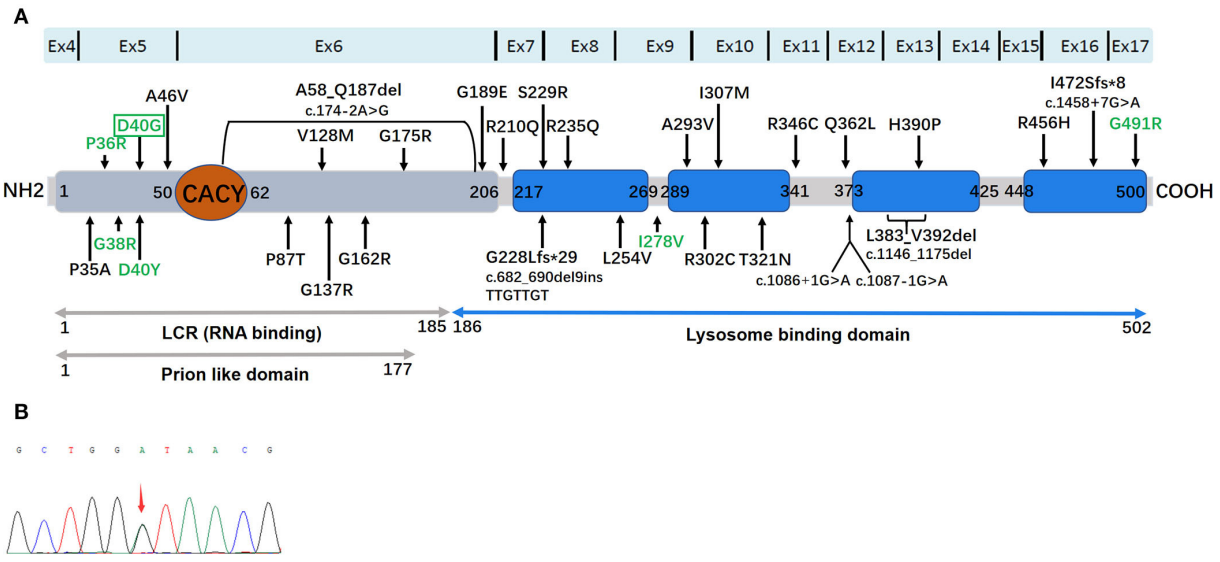

FIGURE 2
(A) The location and distribution of 32 mutations in a 2D schematic representation of the ANXA11 protein. ANXA11 protein and functional domains: a Prion-like domain (gray) was determined by the software PLAAC (Prion Like Amino Acid Composition). RNA (gray) and lysosome (blue) binding domains are represented. The binding site with Calcyclin/S100A6 (CACY) is located at N terminal (orange). The four highly conserved annexin domains are represented by blue square. Reported ALS-related mutations are displayed in black, and ALS/ALS-FTD-related mutations pointed by green. D4OG was detected in the present study are displayed in green by green box. **(B)** Sequence chromatograms of polymerase chain reaction (PCR) products show the heterozygous c.119A>G (p.D40G) mutation in this study.

contains the calcyclin binding site (residues 50–62). Calcyclin can mediate ubiquitination and proteasome degradation of many target proteins (27). In total, four conserved annexin domains, including annexin1-4, constitute the conserved C terminus (28).

ANXA11-related ALS was initially identified in 2017 by whole-exome sequencing in 180 sporadic-ALS (SALS) cases and 751 European familial-ALS (FALS) (17). Smith et al. identified six *ANXA11* mutations (G38R, D40G, G175R, G189E, R235Q, and R346C) in 9 patients from 6 families, and 3 SALS cases without FTD. In the aforementioned study, the D40G mutation was found to be the most common mutation. Patients carrying the D40G mutation presented a delayed-onset of classical ALS symptoms, with 5/6 cases having the bulbar-onset disease. Subsequently, a study in a non-Caucasian population supported the pathogenicity of D40G in the *ANXA11* mutation associated with ALS. Of note, a sporadic ALS case was found once in a Chinese mainland cohort of 383 patients with ALS or ALS–FTD (15). There was also another reported study of 500 Korean patients with SALS (16). Liu et al. failed to discover D40G; instead, they found two rare heterozygous missense variants, namely, c.878C>T (p.A293V) and c.921C>G (p.I307M), in another Chinese cohort with 434 patients with SALS and 50 patients who had the index FALS (24). If the results of the two Chinese cohorts are combined, the D40G mutation rate is rarely low (0.12%, 1/867) in the Chinese patients with ALS or ALS–FTD. The aforementioned results suggest that p.D40G mutation is not the primary cause of ALS in the Chinese population (24).

According to the functional analysis, p.D40G being located near the calcyclin-binding region could cause abnormal binding of calcyclin. Analyses from a postmortem p.D40G ALS case showed profuse annexin A11-positive aggregates in neurons and neuropil of the neocortex and hippocampus, and motor neurons of the spinal cord (17).

In the current study, patients with the same D40G mutation have different clinical symptoms: (1) five of six European patients and one Korean patient who carried the mutation initially showed difficulty in swallowing and speaking (bulbar-onset ALS) (17); (2) a Chinese patient initially displayed left arm weakness at the age of 59 years (15); (3) in the present study, proband 1 with the *ANXA11* p.D40G mutation initially presented abnormal behaviors, executive deficits, and anomia, and later progressed to classic upper motor nervous system damage in the bulbar and limbs. MRI showed significant bilateral temporal lobe atrophy and bilateral signal hyperintensities along the corticospinal tracts. The patient was diagnosed with ALS with bv-FTD. To our knowledge, this study is the first to associate the D40G mutation with

ALS–FTD. Our results provided more genetic support for ALS and FTD.

Reviewing the literature, the spectrum of genotypes and phenotypes associated with *ANXA11*-related diseases has expanded as follows: (14–26) (i) late-onset or early-onset ALS (black mutations in Figure 2); (ii) ALS with FTD (P36R, G38R, D40Y, D40G, I278V, and G491R); (iii) inclusion body myopathy (hIBM), isolated or in combination with ALS/FTD (D40Y). In addition, the ordinary single nucleotide polymorphism (rs1049550, C>T, p.R230C, and MAF 0.44) in *ANXA11* may enhance the risk of sarcoidosis (29). Furthermore, the rs1049550T in the *ANXA11* allele plays a protective role for sarcoidosis in the Chinese Han nationality (30). Like other multisystem proteinopathies (MSP), *ANXA11*-related disorders possess a high clinical heterogeneity (Table 2), suggesting that diverse phenotypes driven by the *ANXA11* mutations require long-term patient follow-ups. Of the six mutations, four mutations that were related to the ALS–FTD phenotype were clustered in *ANXA11* within the long N terminus. The P36R, G38R, D40Y, and D40G mutations are near the calcyclin-binding domain in annexin 11, indicating the functional importance of this region. We know that calcyclin forms a regulatory complex with the calcyclin-binding protein (CACYBP) and RING-type E3 ubiquitin ligase SIAH-1, thereby regulating the ubiquitination and degradation of many proteins, including β-catenin (27). Therefore, calcyclin plays a critical role in proteostasis. However, the pathogenetic mechanism of *ANXA11* mutations leading to ALS–FTD is unclear. Teyssou et al. performed the neuropathological analysis for the G38R case and revealed that FTLD–TDP type A allocations were elicited by the deposition of a mass of TDP-43 lesions in the cortex (31). In patients with ALS, TDP-43 lesion allocations are common because it is associated with a pure FTD phenotype or behavior, related to non-fluent aphasia, or linked to the *GRN or C9orf72* mutation (32). Currently, *in vivo* and *in vitro* experiments are warranted to further this area of research.

In conclusion, this study confirmed the essential role of *ANXA11* mutations in ALS and ALS–FTD. Our results enhanced the understanding of the clinical spectrum and the underlying mechanisms of *ANXA11*-related diseases, including typical ALS, hIBM, FTD, and their combinations.

Data availability statement

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found at: National Center for Biotechnology Information (NCBI) BioProject, https://www.ncbi.nlm.nih.gov/bioproject/, PRJNA832024.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of China-Japan Friendship Hospital (2021-1-Y0). The patients/participants provided their written informed consent to participate in this study.

Author contributions

YW, XD, and DP designed the study. YW, XD, XZho, RW, and DP contributed patient material and clinical data. XW, ZC, XZho, ZZ, XZha, and YS carried out the experiments. YW, XD, DP, and RW analyzed and interpreted the data. YW and XD wrote the manuscript. All authors have made significant contributions and have approved the final version of this manuscript.

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Conflict of interest

Authors ZC and XW are employed by Running Gene Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Dyslexia and dysgraphia of primary progressive aphasia in Chinese: A systematic review

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Introduction: Currently, little is known about Chinese-speaking primary progressive aphasia (PPA) patients compared to patients who speak Indo-European languages. We examined the demographics and clinical manifestations, particularly reading and writing characteristics, of Chinese patients with PPA over the last two decades to establish a comprehensive profile and improve diagnosis and care.

Methods: We reviewed the demographic features, clinical manifestations, and radiological features of Chinese-speaking PPA patients from 56 articles published since 1994. We then summarized the specific reading and writing errors of Chinese-speaking patients.

Results: The average age of onset for Chinese-speaking patients was in their early 60's, and there were slightly more male patients than female patients. The core symptoms and images of Chinese-speaking patients were similar to those of patients who speak Indo-European languages. Reading and writing error patterns differed due to Chinese's distinct tone and orthography. The types of reading errors reported in Chinese-speaking patients with PPA included tonal errors, regularization errors, visually related errors, semantic errors, phonological errors, unrelated errors, and non-response. Among these errors, regularization errors were the most common in semantic variant PPA, and tonal errors were specific to Chinese. Writing errors mainly consisted of non-character errors (stroke, radical/component, visual, pictograph, dyskinetic errors, and spatial errors), phonologically plausible errors, orthographically similar errors, semantic errors, compound word errors, sequence errors, unrelated errors, and non-response.

Conclusion: This paper provides the latest comprehensive demographic information and unique presentations on the reading and writing of Chinese-speaking patients with PPA. More detailed studies are needed to address the frequency of errors in reading and writing and their anatomical substrates.

KEYWORDS

primary progressive aphasia, Chinese-speaking patients, reading errors, tonal errors, writing errors

Introduction

Primary progressive aphasia (PPA) is a clinical syndrome that mainly impairs language function and results from the selective neurodegeneration of the language network. Deficits in language are insidious and progress gradually, presenting as the most prominent clinical feature in the absence of marked impairments in other cognitive and behavioral domains at symptom onset and in the initial phases of the disease (1, 2). According to language phenotype, imaging, and pathology, PPA has been categorized into three types: (1) the non-fluent/agrammatic variant (nfvPPA), which is characterized by agrammatism in language production and effortful speech, and predominant left posterior fronto-insular atrophy/hypometabolism; (2) the semantic variant (svPPA), which is characterized by anomia and single-word comprehension deficits, and predominant anterior temporal lobe atrophy/hypometabolism; and (3) the logopenic variant (lvPPA), which has remarkable features of word retrieval and sentence repetition deficits, and predominant left posterior perisylvian or parietal atrophy/hypometabolism (2).

To date, most studies on PPA have focused on patients speaking Indo-European languages, while there is limited knowledge of the presentations of patients using Chinese, a logographical group of languages completely different from alphabetic languages. In addition, studies on patients using Chinese have been confined to case reports and retrospective studies with small sample sizes. Since PPA is a heterogeneous group of neurodegenerative diseases that selectively damage the language network in the brain, it is reasonable to question whether differences in ethnicities and languages have an impact on the prevalence and manifestations of PPA. To refine the clinical practice paradigm worldwide and pave the way for prompt diagnosis and comprehensive management, it is crucial to incorporate other languages into PPA research. Therefore, we aimed to summarize the demographic data, clinical manifestations, neuropsychological test results, and neuroradiological features of Chinese speakers with PPA from the 56 articles published since 1994 to describe the profile of PPA in Chinese speakers. A review article about the demographics of Chinese patients with frontotemporal dementia (FTD) published in 2012 included 14 patients with nfvPPA and svPPA (3). Here, we provide an up-to-date and comprehensive systematic review of the characteristics of all the three types of PPA in Chinese speakers. Furthermore, we observed that Chinese patients with PPA exhibit some specific reading and writing errors not observed in the Indo-European languages due to the presence of tone and logographic orthography in Chinese (4–11), which will be detailed here.

Chinese tone and script

The syllable of standard Chinese pronunciation, also known as Chinese Pinyin, is the basic unit of standard Chinese phonetic structure. In general, a Chinese character represents a syllable. A syllable consists of three parts: the initial, the final, and the tone. "Initial" and "final" are terms used in ancient Chinese studies, and they only exist in syllables, where they are assigned according to their position. The component of a syllable before the vowel is termed the initial, which refers to the consonants at the beginning of the syllable. The final is the part of a syllable after the initial, consisting of "one to three vowels" or "vowels plus nasal consonants." For example, "nian" is a syllable in standard Chinese, wherein "n" is the initial and "ian" is the final. Tone is the pitch change attached to the initial-final structure, which plays a role in discriminating semantics. There are four tones in standard Chinese: a high-level tone (Tone 1), a mid-rising tone (Tone 2), a low falling-rising tone (Tone 3), and a high-falling tone (Tone 4). For example, the same initial-final structure "nian" signifies different meanings in different tones: with tone 1 it means to pick up (拈niān), with tone 2 it means year (年nián), with tone 3 it means to oust (撵niǎn), and with tone 4 it means to miss (念niàn).

Chinese characters are ideograms and there are no phonograms or grapheme-phoneme correspondence rules in Chinese (12–15). There are around 13,000 Chinese characters in the most widely used modern Chinese dictionaries; nevertheless, on average, roughly 15 characters share the same pronunciation, which are known as homophones (Standards Press of China, 1994). The majority of Chinese words are compound words and are made up of two characters (74%), which effectively eliminates the ambiguity caused by homophones (14). Chinese characters are square-shaped fonts that can be divided into single-component and compound characters, based on their structure. Chinese characters generated directly by the spatial arrangement of strokes are known as single-component characters, which evolved from pictures and signs. For example, the character "口" (kǒu/mouth) looks like a mouth in appearance. When a Chinese stroke "horizontal" (一) which indicates "speech" is added in the middle of "口," it is written as the character "曰" (yūe) and means "to say." After modification of the forms and structures, most single-component characters have been used as Chinese radicals to form compound characters. Two or more single-component characters can be combined according to their meaning to form an associative compound. For example, the combination of "不" (bù/not) and "正" (zhèng/straight) can form "歪" (wāi) to represent "crooked." In addition, more than 80% of commonly used modern Chinese characters are composed of a semantic radical that provides clues to the general meaning category and a phonetic radical that indicates how the character is to be pronounced (15, 16). This is the so-called pictophonetic character. For example, using "木" (mù/wood) as a semantic radical can form characters related to trees such as "桃" (peach), "梅" (plum), "梨" (pear), and the phonetic radical "冈" (gāng/ridge) can form characters with the same pronunciation "gāng" such as "刚" (solid), "岗" (ridge), "钢" (steel). However, because of the historical evolution of phonology and semantics, ∼13% of semantic

radicals have lost their ideographic function, and only ∼37.51% of pictophonetic characters have the same pronunciation as their phonetic radicals and are considered regular characters (17). Conversely, irregular characters have different tones, finals, or are wholly unrelated to their phonetic radicals. Radicals are divided into smaller and indivisible units for character font processing based on visual-spatial/motoric units, that is, components/logographemes (14). For example, the Chinese character "想" (xiǎng/think) consists of two radicals, "相" and "心," which can be further broken down into three components "木," "目," and "心." A Chinese character usually represents a syllable and a Chinese morpheme, forming the characteristic unity of shape, sound, and meaning that Chinese characters have.

Methods

Search strategy

We conducted a systematic review in accordance with the Preferred Reporting Items of Systematic Reviews and Meta-Analysis (PRISMA) guidelines. We systematically searched PubMed, Web of Science, and the Chinese medical databases Wan Fang Database and China National Knowledge Infrastructure (CNKI) to locate all case reports, case series, and treatises on PPA that have been published since 1994. Keywords used for retrieval included "primary progressive aphasia," "progressive non-fluent aphasia," "semantic dementia," "logopenic aphasia" and specified terms like "Chinese," "China," and "Cantonese." Two authors (JL and SO) independently assessed the definitions of PPA. All articles were read carefully and the reference lists were scanned for potential cases to include.

Selection criteria

The cases included in this review were required to fulfill the basic PPA criteria proposed by Mesulam (18, 19). We adopted the subtype classification of cases proposed by the original authors. Cases with a definitive diagnosis of PPA but no reported subtypes were reclassified according to the consensus criteria published in 2011 (2). The exclusion criteria were as follows: (1) the patients were not of Chinese ethnicity; (2) articles with neither demographic nor language data; (3) the study subjects were classified as FTD (no subtype classification), Amyotrophic lateral sclerosis (ALS), ALS-FTD (no subtype classification), and other diseases, or the variants of cases were behavioral variant frontotemporal dementia (bvFTD) and right temporal lobe variant of semantic dementia (RTLV); (4) cases with a diagnosis of PPA published between 1994 and 2011, which did not contain sufficient information to support subtype classification; and (5) articles with questionable diagnosis and unclear data. If the same cases were reported in several publications, they were counted only once. Overall, 180 cases from 56 publications were included in this systematic review (Figure 1) (20).

Data collection and analysis

Demographic and clinical information collected included age at onset and recruitment, sex, disease duration, level of education, clinical manifestations of cognitive function, psychobehavioral symptoms, neurological signs (parkinsonism, supranuclear gaze palsy, motor neuron features), and language features (reading, writing, spontaneous speech, repetition, single-word and sentence comprehension, confrontation naming, and grammar), Mini-Mental State Examination (MMSE) scores, and neuroimaging. Cases that underwent examinations on three or more of the six language domains (reading, writing, repetition, comprehension, naming, and grammar) were included in the analysis of clinical manifestations. All analyses were performed using the R software (version 3.6.2). Continuous variables are described using mean (standard deviation, SD) or median [interquartile range, IQR] with analysis of variance (ANOVA) or Kruskal-Wallis H test to compare differences between groups. Categorical variables are described using the number of cases (percentage). Differences in distribution between the groups were compared using the corrected chi-square test or Fisher's exact test. Pairwise comparisons between groups for continuous variables were performed using the SNK-q test (normal distribution) or Benjamini and Hochberg (BH) adjusted Dunn's multiple comparisons (non-normal distribution), and pairwise comparisons between categorical variables were performed using the chi-squared partition. An FDR-adjusted p-value was used for *post-hoc* comparisons.

Results

Demographic features and cognitive assessments

Table 1 shows the demographic data and neuropsychological test results of the nfvPPA, svPPA, lvPPA groups. Sixteen patients (five with nfvPPA, four with svPPA, and seven with lvPPA) with detailed clinical manifestations lacked respective demographic data and thus were not included here. In addition, three cases were classified as unclassified PPA (21, 22). A total of 161 patients were therefore included in Table 1. The three groups were comparable in terms of sex, age of onset and recruitment,

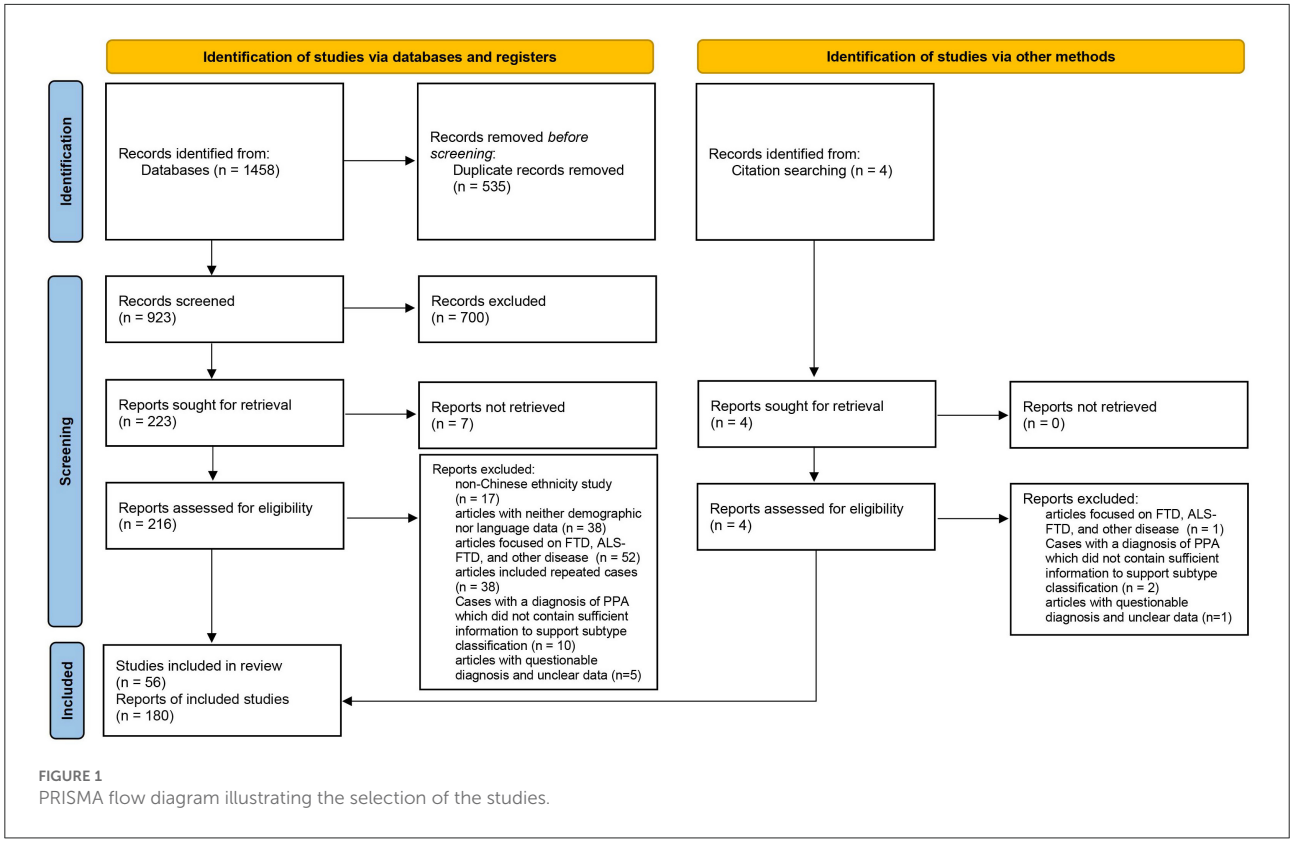

FIGURE 1
PRISMA flow diagram illustrating the selection of the studies.

disease duration, educational level, and general cognitive assessment scores.

Features of language and other cognitive impairments

Table 2 shows features of language impairments and other cognitive and behavioral domains in each PPA type. Thirty-three patients (4 nfvPPA, 29 svPPA) with detailed demographic data but no comprehensive clinical manifestations were not included here.

All patients with nfvPPA had early onset non-fluent expression difficulty with reduced, slow, effortful, and halting speech. Some also exhibited sound errors, abnormal intonation, impaired volume control, and speech that lacked information. The nfvPPA group had more marked pronunciation distortions and impaired grammatical comprehension than the other two groups. Patients with nfvPPA had more prominent phonological paraphasia, repetition impairments, and agrammatism than patients with svPPA. The majority of patients with nfvPPA showed syntactic errors, such as short sentences, simple or impaired structures, lack of function words, and word order errors.

Patients with svPPA spoke fluently and exhibited anomia, impaired word comprehension, and word-finding difficulties. They lost semantic knowledge of nouns, verbs, color, and shape at an early stage, and some of them showed category-specific semantic deficits (23). Therefore, they lacked notional words and spoke with empty contents. It should be noted that the auditory comprehension impairments of svPPA were mainly for words, and those of nfvPPA and lvPPA were mainly for sentences. Patients with svPPA had more naming errors than patients with nfvPPA. Besides, one svPPA patient made word order errors in writing, such as writing "银行" (bank) as "行银." A small percentage of patients' initial symptoms also included facial agnosia or memory decline.

Patients with lvPPA initially presented with word-finding difficulties, naming errors, and frequent phonological paraphasia, with about half of them having less fluent speech. Seven patients with lvPPA exhibited simply structured spontaneous speech. Patients with lvPPA had more prominent phonological paraphasia, repetition impairments, and agrammatism than patients with svPPA.

There were no significant differences in word-finding difficulties, semantic paraphasia, dyslexia, dysgraphia, episodic

TABLE 1 Demographic characteristics and clinical scores in patients with PPA variants.

Variables	ALL	nfvPPA	svPPA	lvPPA	*p*-Value
	n = 161	*n* = 22	*n* = 123	*n* = 16	
Sex					0.443
Male	93 (57.8%)	14 (63.6%)	72 (58.5%)	7 (43.8%)	
Female	68 (42.2%)	8 (36.4%)	51 (41.5%)	9 (56.2%)	
Age at onset (years)	61.5 (8.18)	61.6 (10.5)	61.9 (8.07)	58.4 (4.22)	0.272
Age at recruitment (years)	64.4 (8.43)	64.6 (10.4)	64.7 (8.39)[a]	61.8 (5.29)	0.411
Disease duration (years)	3.00 [2.00;4.00]	3.00 [2.00;4.00]	3.00 [2.00;4.00][a]	2.00 [1.75;5.00]	0.962
Educational level					0.547
Illiteracy	5 (3.36%)	0 (0.00%)	5 (4.31%)	0 (0.00%)	
Primary school	18 (12.1%)	4 (23.5%)	13 (11.2%)	1 (6.25%)	
Secondary school	76 (51.0%)	7 (41.2%)	62 (53.4%)	7 (43.8%)	
College	50 (33.6%)	6 (35.3%)	36 (31.0%)	8 (50.0%)	
MMSE scores (range 0–30)	18.0 [11.0;23.0][b]	20.5 [7.50;25.8][c]	18.0 [11.5;23.0][d]	13.0 [9.00;20.2][e]	0.303

Data are represented as mean (SD), median [IQR], or n (%). nfvPPA, non-fluent variant primary progressive aphasia; svPPA, semantic variant primary progressive aphasia; lvPPA, logopenic variant primary progressive aphasia; MMSE, Mini-Mental State Examination.
[a]n = 120, [b]n = 123, [c]n = 18, [d]n = 91, [e]n = 14.

TABLE 2 Clinical manifestations of patients with PPA variants.

Variables	nfvPPA	svPPA	lvPPA	*P*-value	Adj.p[a]	Adj.p[b]	Adj.p[c]
	n = 23	*n* = 98	*n* = 23				
Pronunciation distortion	12/22 (54.5%)	1/56 (1.8%)	3/22 (13.6%)	**<0.001**[a,b]	<0.001	<0.05	0.118
Phonological paraphasia	9/22 (40.9%)	0/56 (0.0%)	14/22 (63.6%)	**<0.001**[a,c]	<0.001	0.227	<0.001
Word finding difficulties	8/22 (36.4%)	32/56 (57.1%)	13/22 (59.1%)	0.206			
Semantic paraphasia	3/22 (13.6%)	9/56 (16.1%)	3/22 (13.6%)	0.944			
Impaired auditory comprehension	17/23 (73.9%)	74/79 (93.7%)	18/23 (78.3%)	**0.016**[a]	0.063	1.000	0.110
Naming errors	18/22 (81.8%)	98/98 (100.0%)	22/23 (95.7%)	**<0.001**[a]	<0.001	0.428	0.428
Repetition impairments	20/21 (95.2%)	38/84 (45.2%)	21/21 (100.0%)	**<0.001**[a,c]	<0.001	1.000	<0.001
Dyslexia	11/16 (68.8%)	66/86 (76.7%)	12/20 (60.0%)	0.291			
Dysgraphia	15/20 (75.0%)	39/60 (65.0%)	7/13 (53.8%)	0.452			
Agrammatism in speech	14/14 (100.0%)	1/23 (4.4%)	7/10 (70.0%)	**<0.001**[a,c]	<0.001	0.118	<0.001
Impaired grammatical comprehension	17/19 (89.5%)	0/81 (0.0%)	0/10 (0.0%)	**<0.001**[a,b]	<0.001	<0.001	-
Episodic memory loss	10/17 (58.8%)	47/82 (57.3%)	13/15 (86.7%)	0.097			
BPSD	9/18 (50.0%)	61/78 (78.2%)	11/16 (68.8%)	0.052			
Neurological positive signs	5/10 (50.0%)	2/25 (8.00%)	1/12 (8.3%)	**0.008**	0.058	0.132	1.000

Data are represented as %. nfvPPA, non-fluent variant primary progressive aphasia; svPPA, semantic variant primary progressive aphasia; lvPPA, logopenic variant primary progressive aphasia; BPSD, behavioral and psychological symptoms of dementia.
Bold values denote statistical significance at the $p < 0.05$ level.
Significantly different: [a]nfvPPA vs. svPPA; [b]nfvPPA vs. lvPPA; [c]svPPA vs. lvPPA.
For post-hoc comparisons, significance is set at $p < 0.05$.

memory loss, and behavioral and psychological symptoms of dementia (BPSD) among the three groups, and no significant difference in impaired auditory comprehension and neurological positive signs in the multiple comparisons analysis.

Neuroimaging features in each PPA type

The neuroimaging results are shown in the Supplementary Table 1. Twenty-seven patients (six with nfvPPA, fourteen with svPPA, and seven with lvPPA) without images were not included here. Approximately half of the nfvPPA patients presented with left frontal and temporal lobes atrophy/hypometabolism, which was much greater in the left inferior frontal gyrus (4, 24, 25). The other half showed bilateral asymmetric atrophy/hypometabolism of the frontal and temporal lobes, which was more pronounced on the left side. Lesions in the left temporal lobe, particularly the temporal pole, were more common in patients with svPPA, with 14

patients having more severe lesions on the right side. The most prominent area of involvement in patients with lvPPA was the left temporoparietal area.

Features of reading impairments in Chinese PPA

The types of reading errors are listed in Table 3. The types of reading errors reported in Chinese-speaking patients with PPA included tonal errors, regularization errors, visually related errors, semantic errors, phonological errors, unrelated errors, and non-response. Because there were not many detailed reports, we could only provide the type of errors, not the frequency. The information provided by the case-control studies that were not included is shown in Table 3.

The study of reading in svPPA was the most comprehensive among the three subtypes. Reading errors in Chinese patients with svPPA can be classified into five types:

(1) Regularization errors: Regularization errors are further classified into two subclasses. The first is called "legitimate alternative reading of components (LARC) errors," which refers to misreading an irregular Chinese character into one of its pronounceable components that is inappropriate for the target character but is legitimate and more typical (16, 26, 27). For example, misreading "腔" (qiāng/cavity) as its phonetic radical "空" (kōng/empty), and misreading "笔" (bǐ/pen) as its semantic radical "毛" (máo/fur) (5, 11). On the other hand, there are more than 250 heteronyms that have more than one pronunciation with their respective meanings in 3,500 commonly used Chinese characters. The second type of regularization error refers to the situation in which patients read the target character as one of its other pronunciations, e.g., the character "的" was pronounced as "dī" when it was supposed to read as "dē" (28).
(2) Visually related errors: Visually related errors occur when the output corresponds to a character that is orthographically similar to the target (29). For instance, misreading "旱" (hàn/drought) as a visually similar character "早" (zǎo/morning) (7).
(3) Semantic errors: Semantic errors occur when the output and target characters are semantically related (15, 30), such as the confusion of "刷" (shuā/brush) and "扫" (sǎo/sweep) (10).
(4) Unrelated errors: For example, "盐" (yán/salt) read as "gui" (created by the patient).
(5) Non-response.

Among these error types, regularization errors were the most common in svPPA. Some patients were able to read characters aloud correctly without comprehending meaning (7), while others showed a better understanding of words/characters and instructions than ability to read them aloud (31). Another interesting phenomenon was that some patients directly judged the meaning of the Chinese characters as the meaning of their radicals.

Errors reported in patients with nfvPPA included tonal errors, phonological errors, and non-response. Tonal errors signify that the pronunciation of the output and target differed only in tone (9). For example, "年" (nián/year) read as "念" (niàn/miss). Compared with controls, nfvPPA patients performed very poorly in all tone production tasks, such as reading out loud a set of characters with similar initial-final structure but different tones. Phonological errors mean that the response and the target share at least half of the phonetic features (initial-final structures) (29). For example, "年" (nián/year) read as "娘" (niáng/mother). Tonal errors were unique to Chinese patients with PPA, and patients with nfvPPA tended to make more tonal errors than phonological errors (9). The reading comprehension of words/characters was better than that of sentences. Although some patients failed to read words/characters aloud, they could understand their meanings (4, 25).

Reading errors in patients with lvPPA included visually related errors, semantic errors, and phonological errors (8, 32).

Features of writing impairments in Chinese PPA

Most PPA patients with dysgraphia showed better ability to write their own names and addresses, and copy characters in writing examination, but had difficulties in dictation and spontaneous writing. PPA may only affect sophisticated tasks such as dictation at first, but as the disease progresses, deterioration will become obvious in other tasks. Although the majority of papers have reported PPA patients' writing impairments, thorough reports on error types and probabilities are uncommon.

The types of writing errors are presented in Table 4. The types of writing errors reported in Chinese-speaking patients with PPA included phonologically plausible errors, orthographically similar errors, semantic errors, compound word errors, sequence errors, unrelated errors, non-character errors, and non-response. Overall, writing errors can be classified into two broad categories: non-character responses and incorrect character responses.

Non-character responses mainly include the following 6 types:

(1) Stroke errors are easily identifiable when the target strokes are deleted, added, substituted, and transposed.
(2) Radical/component errors indicate that the target radicals/components have been deleted, added, substituted, and transposed.

TABLE 3 Types of reading errors of patients with PPA variants.

Error type	nfvPPA	svPPA	lvPPA
Tonal errors	++	(++)	(+)
Regularization errors	(+)	++	(+)
Visually related errors	(+)	++	+
Semantic errors	?	+	+
Phonological errors	+	-	+
Unrelated errors	?	+	?
Phonological dyslexia	-	-	-
Non-response	+	+	?

+ =present, ++ =present commonly, -=not present, ?=unclear. Symbols in parentheses represent the information provided by non-included case-control studies. nfvPPA, non-fluent variant primary progressive aphasia; svPPA, semantic variant primary progressive aphasia; lvPPA, logopenic variant primary progressive aphasia.

TABLE 4 Types of writing errors of patients with PPA variants.

Error type	nfvPPA	svPPA	lvPPA
Non-character errors			
Stroke errors	+	+	+
Radical/component errors	+	+	+
Visual errors	+	(+)	+
Pictograph errors	-	+	-
Dyskinetic errors	+	-	+
Spatial errors	+	-	-
Phonologically plausible errors	+	+	+
Orthographically similar errors	+	+	?
Semantic errors	+	+	+
Compound word errors	+	(+)	+
Sequence errors	?	+	?
Unrelated errors	+	+	+
Non-response	+	+	+

+ =present, -=not present, ?=unclear. Symbols in parentheses and the results on lvPPA are based on the findings provided by non-included case-control studies. nfvPPA, non-fluent variant primary progressive aphasia; svPPA, semantic variant primary progressive aphasia; lvPPA, logopenic variant primary progressive aphasia.

(3) Visual errors manifest as errors in which the radicals/components are substituted by non-existent radicals, but the output visually resembles the target.
(4) Pictograph errors refer to the substitution of Chinese characters with pictogram symbols, e.g., a patient drew an umbrella when dictating the character "伞" (umbrella) (7).
(5) Dyskinetic errors are caused by dyskinesia in the writing hand, resulting in relatively intact glyphs with interrupted, incomplete, or disproportionate strokes.
(6) Spatial errors mean that the radical is placed in an inaccurate position, and the spatial position between the radicals is enlarged as if there are two independent Chinese characters.

Incorrect character responses are defined as real characters included in the Modern Chinese dictionary, but not the target character. These can be subdivided into the following six types:

(1) Phonologically plausible errors primarily refer to a writing phenomenon corresponding to surface dyslexia in Indo-European languages, also known as surface dysgraphia, which refers to dictating exception words following sound-to-spelling conversion rules (33). In Chinese, they mainly denote characters that are homophonic or phonologically similar to the target, including those that differ only in their tone. Most of the target characters were replaced by higher frequency characters, e.g., "架" (jià/shelf) was replaced by "价"

(jià/price), and "访" (fǎng/visit) was replaced by "反" (fǎn/contrary) (7, 9).

(2) Orthographically similar errors may be caused by stroke or radical/component errors. For example, the character "月" (moon) was written as "目" (eye) in which a "horizontal" was added, and the compound character "想" (think) was written as "相" (phase), in which the radical "心" (heart) was deleted (7). In addition, characters with similar structures are also part of this range, e.g., "去" (go) and "生" (get) (4).

(3) Semantic errors refer to the output and the target having similar or relevant meanings, such as writing "岁" (age) as "年" (year) (4).

(4) Compound word errors describe errors in which the target is substituted by another character of a compound word. For example, the character "整" was written when patients were asked to write "齐" of the compound word "整齐" (neat) (9).

(5) Sequence errors refer to reversal of the sequence of stroke writing.

(6) Unrelated errors indicate that patients write characters that are not phonologically, orthographically, or semantically similar to the target characters.

Writing errors found in nfvPPA patients comprised phonologically plausible errors, orthographically similar errors, semantic errors, compound word errors, unrelated errors, non-character errors (stroke errors, radical/component errors, visual errors, dyskinetic errors, and spatial errors), and non-response. Patients with svPPA showed phonologically plausible errors, orthographically similar errors, semantic errors, sequence errors, unrelated errors, non-character errors (stroke errors, radical/component errors, pictograph errors), and non-response. Unfortunately, there were no detailed reports of patients with lvPPA amongst the cases studied here.

Discussion

Demographic features of Chinese-speaking PPA

Statistically, the prevalence of PPA is approximately three cases per 100,000 (34, 35), while the prevalence of PPA in China has not yet been reported. The number of patients with svPPA in our study was significantly larger than that of other subtypes. While there is no agreement concerning which subtype of PPA is the most common: a multicenter study from France suggested that the most common subtype is lvPPA (34), whereas nfvPPA predominates in our research in Japan (36). Since our study was more affected by publication bias, in that svPPA patients are more suitable for case reports due to their characteristic language impairments compared to other PPA subtypes, it is preferable to refer to other Chinese case-control or cohort studies. A study from Shanghai included three times more patients with svPPA than with nfvPPA (37). However, a study from North China enrolled the same number of nfvPPA patients as svPPA patients (38), and a Northeast Chinese Master's study included more patients with lvPPA. Thus, we cannot conclude that svPPA is the most common subtype in China. Studies have shown that the typical age of onset of PPA ranges from 50 to 70 years, with an average age of onset in the late fifties and nearly equal prevalence in both sexes (1, 39–41). The mean age of PPA onset in our study was slightly higher, with a slight male predominance.

Features of language and other cognitive impairments in each PPA type

The core symptoms of Chinese patients with PPA were the same as those of Indo-European- and Japanese-speaking patients. Sound-level errors in nfvPPA were caused by both apraxia of speech and/or phonological paraphasia, which is consistent with previous research (42). Impairments in auditory comprehension and repetition in patients with nfvPPA were mainly caused by agrammatism. Moreover, impaired speech motor planning and the subvocal rehearsal component may contribute to repetition deficits. In svPPA, this may be due to the disintegration of semantic representations, and in lvPPA patients, it may have been due to impaired short-term memory and phonological storage (43, 44). Studies have shown varying prevalence and extent of memory deficits for PPA variants, with evidence of widespread episodic memory loss in lvPPA patients (45). In terms of behavior changes, patients with svPPA exhibit significantly more behavioral disturbances than other PPA subtypes, including disinhibition, eating habit changes, stereotyped behavior, and empathy loss (35, 46). In our study, the probability of episodic memory loss did not differ significantly across PPA subtypes, although it was higher in lvPPA patients. In addition, there was no significant difference in BPSD, even though the percentage of neuropsychiatric symptoms in svPPA was higher among the three groups. The fact that less than half of the patients underwent neuropsychological tests, such as the Neuropsychiatric Inventory (NPI) could have influenced the results. Studies in the Indo-European language have shown that agrammatism in patients with nfvPPA is characterized by impaired production of verb inflection and verb argument structure, omission of function words, and reduced grammatical complexity (47, 48). Meanwhile, word order errors due to agrammatism in patients with nfvPPA aid in differential diagnosis (49). In Chinese, there are no restricted morphological changes (such as singular and plural, tense and subject-verb agreement). Therefore, the agrammatic error types in nfvPPA manifest mainly in word order, function words, and complexity in Chinese. The grammar ability of patients with

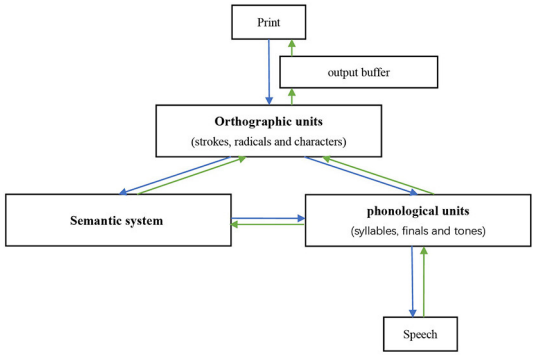

FIGURE 2
The "triangle model" of reading and writing in Chinese.

svPPA has always been considered to be preserved (2, 48). Studies in Indo-European languages have proposed that the reduced performance of svPPA patients in word ordering tasks could be due to word comprehension deficits (49). Accordingly, word order errors in the writing of an svPPA patient in our case were considered be due to impaired semantics. Mild grammatical problems, such as reduced grammatical complexity, were also found in patients with lvPPA, which is consistent with previous studies (47, 50). Each subtype of PPA could show agrammatism, because agrammatism involves various symptoms, such as missing verbs, reduced sentence complexity, and omitted functional words, and is associated with a large neural network involving the left posterior middle temporal gyrus, inferior parietal lobe, inferior frontal gyrus, and their connecting fiber bundles (51–54).

Features of reading and writing impairments in Chinese PPA

The function model of reading and writing in Chinese

To better investigate the potential mechanisms, it is necessary to first introduce the functional models of reading and writing in Chinese, which differ from the dual-route model used in alphabet language (30, 55). The morphemic/syllabic level, rather than the phonemic level, is where Chinese characters map into language (29, 56). Unlike English words, which assemble phonemic units/syllables, the pronunciation of Chinese compound characters does not directly assemble the pronunciation of radicals and components. In other words, radicals/components do not correspond to the sub-syllabic units of phonological representations (57). Due to the above differences and the lack of grapheme-phoneme correspondence rules, a "triangle model" has been put forward, wherein reading in Chinese is proposed to depend on two independent and interrelated pathways (Figure 2): a lexical semantic pathway connecting orthographic units, semantic system, and phonological units, and a non-semantic pathway that contacts all orthographic representations (i.e., strokes, radicals, and characters) to all phonological representations (i.e., syllables, finals, and tones) bypassing the semantic system (13, 26, 30). Phonetic and semantic radicals have been proven to access their phonological and semantic representations in parallel with entire characters (16, 58). The neurocognitive process of writing to dictation is assumed to occur *via* reversal of these pathways (12, 15, 30). After the orthographic representations are retrieved, they are stored in an amodal "output buffer" until they are further processed (14).

The cognitive mechanism of tonal errors

The results showed that patients with nfvPPA presented with tonal errors in reading. Gorno-Tempini et al. observed that svPPA and lvPPA also exhibit tonal dyslexia (59). Patients with nfvPPA tend to make tone substitution errors, while patients with svPPA are prone to regularization errors in serial tone reading tasks (60). Furthermore, the accuracy of serial tone reading was much better in patients with lvPPA than in those with the other two subtypes. In addition, the performance of the tone-word matching test was poor in nfvPPA and svPPA patients, while that in lvPPA patients was relatively preserved (59). For example, patients made mistakes when they were asked to select the target that corresponded to the auditory stimulus from four Chinese characters with the same initial-final structure but different tones. Tonal errors are unique to Chinese patients and are not found in Japanese or English-speaking patients. Therefore, tonal tasks can be used as a potential diagnostic tool for Chinese-speaking PPA patients (9, 59, 60).

Pitch changes in tone are related to the anatomical properties of the speakers' vocal folds and larynx, and the fundamental frequency of vocal cord vibration per unit time. Due to the high prevalence of motor speech disorders, particularly apraxia of speech, in nfvPPA patients, it is not surprising that Chinese nfvPPA patients presented with tone dyslexia (61–63). For patients with svPPA and lvPPA, tonal errors were more likely to be caused by disorganized phonological codes. In terms of the triangle model, tonal errors are likely to result from a disruption of phonological units (12, 30). Law et al. proposed a phonology structure in which phonological representations have a multitiered form (12). Segmental features (i.e., consonants and vowels) and suprasegmental features (i.e., tones) are at the tiers of separation and generate connections independently of each other. Thus, once tonal information is disrupted, the suprasegmental tier will disassociate from the segmental tier, and a highly similar and intact representation with the same initial-final structure but different tone will be output or mapped into orthographic units instead of the impaired target.

The cognitive mechanism of errors on orthography

The results showed that both svPPA and lvPPA patients had visually related errors in reading. Patients with nfvPPA showed visual errors in writing, and orthographically similar errors, stroke errors, and radical/component errors were present in both nfvPPA and svPPA patients. In addition, the majority of stroke, radical/component, and visual errors retained the inherent configuration of Chinese characters. It is important to note that all three subtypes show these types of errors in reading and writing in practice. Whereas, visually related errors were more prominent in svPPA, radical/component errors were more common in nfvPPA, and visual errors and stroke errors were much more common in lvPPA (64, 65). In fact, visually related errors were also reported in Japanese patients with svPPA (66), and all three subtypes showed such writing errors in kanji character in our clinical work. Therefore, these types of errors are common in patients with PPA using logographic orthography.

The triangle model could account for visually related errors by implying that the mapping between print and orthographic units, or orthographic units *per se*, was faulty. Law et al. suggested that orthographic representations include not only the identities of the components and radicals, but also information on the structure of characters, perhaps in the form of structural templates or specification of position for each constituent (29). When the identity information of one of the constituents is selectively impaired, the system may fill in the missing information with another constituent based on intact structural information, such that the overall configuration of the character remains unaffected. Errors in writing involve additional speculations. Chinese characters are more dependent on orthographic working memory than English words due to their visuospatial intricacy (64). Therefore, disorders of the "output buffer" which contains graphic information (shape and/or stroke features) may result in these errors (14). Studies have shown that the dictation accuracy of Chinese patients with lvPPA decreases with increasing stroke numbers, and the left lingual gyrus is involved in this possess, which further supports this viewpoint (64).

The cognitive mechanism of regularization errors and phonologically plausible errors

The results showed that svPPA patients presented with regularization errors, and phonologically plausible errors were present in nfvPPA and svPPA patients. Studies have shown that regularization errors were found in all three subtypes of PPA, whereas they were more marked in svPPA (7, 65, 67). In addition, phonologically plausible errors were also present in lvPPA patients (64).

Similar to English- and Japanese-speaking svPPA patients characterized by surface dyslexia (2, 63, 66, 68–70), Chinese svPPA patients tend to make regularization errors in their reading. However, there was a little difference between all three. In English, patients assemble common pronunciations of phonemes or syllables directly to read irregular words, e.g., reading pint /paInt/ as /pInt/. In Japanese, about two-thirds of kanji characters have two or more different pronunciations, and the correct pronunciation depends on word/character collocation. Surface dyslexics assign a pronunciation that is wrong for the target word but legitimate for that character in other words (27). For example, reading "海老" (shrimp) /ebi/ as /kai/ and /rou/. In Chinese, regularization errors are divided into two subclasses. The second type manifests itself in the same way as surface dyslexia in Japanese; in both cases, the target character is read as one of its other pronunciations. The first (LARC errors) is different in that Chinese characters are read as one of its pronounceable radicals or components, that is, a constituent of the character font.

In terms of agraphia, patients with svPPA using Indo-European languages and Japanese tend to have phonologically plausible errors/surface dysgraphia (33, 68, 71, 72). Similarly, phonologically plausible errors are also frequently observed in Chinese svPPA (64). Phonologically plausible errors in Chinese can be classified into three types: errors that are homophonically or phonologically similar to the target, and errors that differ only in tone from the target. The first type is similar to surface dysgraphia in Japanese, in that both produce high-frequency and more common homophones at the lexical level. Instead of the phonologic regularity effect, writing accuracy in Chinese svPPA and lvPPA patients was associated with homophone density (64). Meanwhile, the performance of Indo-European-speaking patients exhibited a difference in that they dictated exception words following the sound-to-spelling conversion rules at the sublexical level, e.g., dictating "pint" as "paint."

The triangle model suggests that regularization errors may be caused by selective lexical-semantic pathway impairment (13, 73). Radicals/components that emerge more frequently than entire characters might dominate phonological computation through the lexically mediated non-semantic pathway instead of the whole characters in the absence of adequate semantic constraints (15, 26, 30). Since Chinese is an opaque language with many homophones, the semantic system aids in eliminating ambiguity in orthographic output selection (74). However, phonologically plausible errors may occur when the impaired semantic system fail to provide appropriate semantic guidance (12, 30) and when relatively preserved phonological processing is overused (64). However, the essence of regularization errors and phonologically plausible errors in PPA patients who speak Chinese, Japanese, and English, is the loss of

semantic knowledge; phenotypic differences only exist because of language differences.

Other reading errors

Compound word errors were observed in the patients with nfvPPA. Studies have shown that they can arise in all three subtypes, but more prominently in patients with nfvPPA. Due to the use of abundant compound words in Chinese and a correlation with the bilateral orbitofrontal gyrus, such errors may be secondary to the inability to inhibit the other characters of the two-character compound words (64). Furthermore, unlike English-speaking lvPPA patients that are characterized by phonological dyslexia, Chinese-speaking lvPPA patients were competent in reading pictophonetic pseudowords, such as the pseudoword "木冈" which consists of a semantic radical "木" and a phonetic radical "冈" (65). There are two possible reasons for this. First, pseudowords are made up of a phonetic radical and a semantic radical, and their pronunciation is consistent with that of the phonetic radical without the use of grapheme-phoneme correspondence rules. Therefore, patients can read this pseudoword depending on the phonetic radical. Second, Chinese characters are highly concentrated symbols with sound, form, and meaning, and are not susceptible to such errors. Finally, in addition to the error types mentioned in our results, Tee et al. also reported other rare errors such as phonetic radical errors, neographism, and perseveration dysgraphia (64).

Limitation

Due to the inevitability of incomplete or missing data in a retrospective study, we can only provide the frequency of symptoms rather than the degree. The distribution of cases of each subtype was also affected by publication bias. In addition, we discovered that there are no unified language tests for PPA in China. In addition to the most commonly used Aphasia Battery of Chinese (ABC), researchers have adopted other scales such as the Western Aphasia Battery (WAB) and the Boston Diagnostic Aphasia Examination (BDAE). It is unavoidable to miss some of the less obvious symptoms owing to the lack of unified linguistic assessment tools and scoring criteria for patients with PPA. The absence of a standard impedes the comparability of patients from different studies for clinical and research purposes. Furthermore, there are no restricted morphological changes in Chinese, and word order and function words are the main ways to express grammatical relations. Therefore, it is more difficult to identify grammatical anomalies in Chinese than in Indo-European languages. Meanwhile, an anagram task (75), which is used in patients with severely reduced English language production, is absent from the Chinese grammatical assessment. Consequently, the description of agrammatism in articles is sometimes vague or lacking. Finally, none of the patients we gathered underwent pathological investigation, and fewer than 10 underwent lumbar puncture; therefore, the neuropathological changes that could result in PPA remain unknown. In conclusion, multicenter and multiregional research is expected to provide more comprehensive and detailed clinical data by employing a unified language task, which includes a detailed grammar examination.

Conclusion

This paper provides the latest comprehensive demographic information on Chinese-speaking patients with PPA, summarizes their unique presentations in reading and writing, and investigates the underlying mechanisms for understanding PPA features in languages other than Indo-European languages. This review emphasizes the importance of establishing a standard diagnostic process across multicenter sites to form a large cohort, gain a more complete understanding of the full spectrum of PPA in Chinese patients, and improve diagnostic precision. More studies are expected to be conducted in Chinese-speaking patients with PPA to clarify the error frequency in reading and writing and their anatomical substrates.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

KS and JL conceived the presented idea and framework for the systematic review. JL conducted the search for articles in consultation with KS and made the table and completed the PRISMA diagram. JL and SO analyzed the articles in consultation with KS. JL drafted the manuscript and KS and SO were involved in the planning of the manuscript. KS, SO, NK, and SK revised and provided feedback for the manuscript. All authors revised and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1025660/full#supplementary-material

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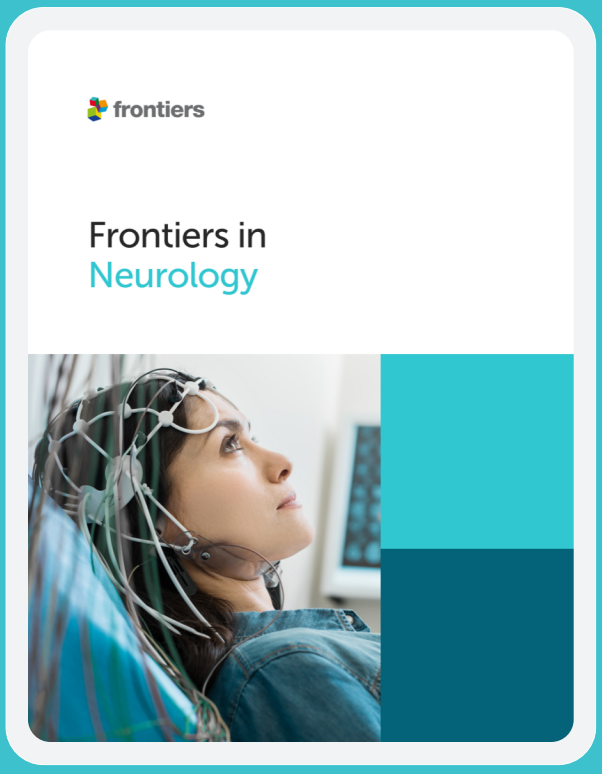

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