DEMENTIA AND NEURODEGENERATIVE DISEASES EDITOR'S PICK 2021

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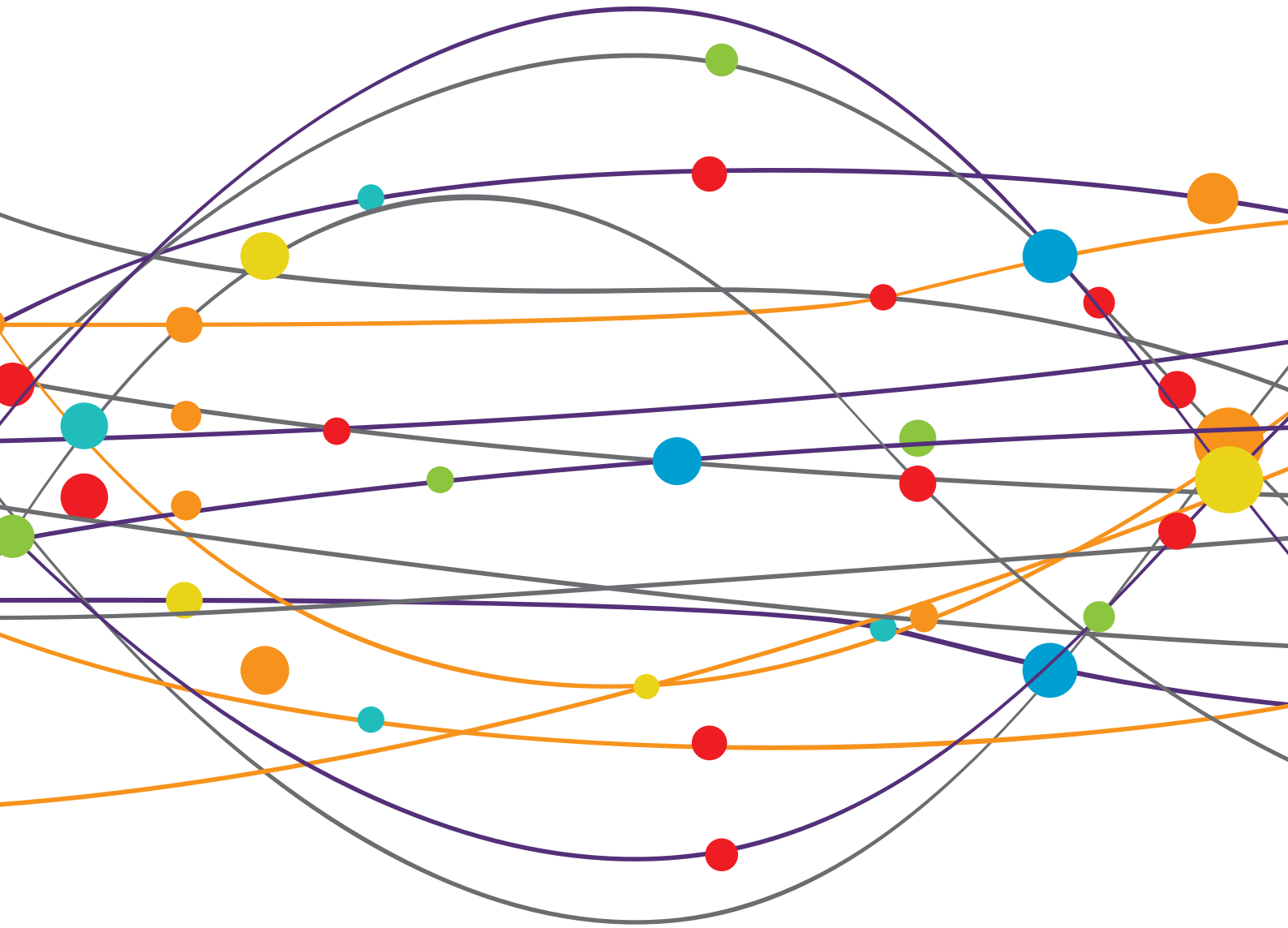

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DEMENTIA AND NEURODEGENERATIVE DISEASES EDITOR'S PICK 2021

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Modifiable Lifestyle Factors and Cognitive Function in Older People: A Cross-Sectional Observational Study

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Background: The development of evidence-based interventions for delaying or preventing cognitive impairment is an important challenge. Most previous studies using self-report questionnaires face problems with reliability and consistency due to recall bias or misclassification among older people. Therefore, objective measurement of lifestyle components is needed to confirm the relationships between lifestyle factors and cognitive function.

Aims: The current study examined the relationship between lifestyle factors collected with wearable sensors and cognitive function among community-dwelling older people using machine learning.

Methods: In total, 855 participants (mean age: 73.8 years) wore a wristband sensor for 7.8 days on average every 3 months. Various lifestyle parameters were measured, including walking steps, conversation time, total sleep time (TST), sleep efficiency, time awake after sleep onset, awakening count, napping time, and heart rate. Random forest (RF) regression analysis was used to examine the relationships between total daily sensing data and Mini-Mental State Examination (MMSE) scores. Confounding factor analysis was conducted with models that were adjusted and unadjusted for demographic and vascular risk factors, and selected variables were assessed as risk and protective factors using partial dependence plots (PDPs).

Results: Lifestyle data were collected for 31.3 ± 7.1 days per year using wristband sensors. RF regression analysis adjusted for age, gender, and education levels selected four variables, including number of walking steps, conversation time, TST, and heart rate. Moreover, walking steps, conversation time, and heart rate remained after RF regression analysis adjusted for demographic and vascular risk factors. Number of walking steps, conversation time, and heart rate were categorized as protective factors, whereas TST was categorized as a risk factor for cognitive function. Although PDPs of number of walking steps and heart rate revealed continuously increased MMSE scores, those of conversation time and TST and revealed that the tendency in the graph was reversed at the boundary of a particular threshold (321.1 min for conversation time, 434.1 min for TST).

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Conclusions: Lifestyle factors, such as physical activity, sleep, and social activity appear to be associated with cognitive function among older people. Physical activity and appropriate durations of sleep and conversation are important for cognitive function.

Keywords: cross-sectional study, lifestyle factors, cognitive function, wearable sensor, mini-mental state examination, random forest regression analysis

INTRODUCTION

Dementia is a major public health issue worldwide, with a serious burden for patients, caregivers, and society, as well as substantial economic impacts (1). Although the prevalence of late-life cognitive impairment and dementia are expected to increase in future, effective disease-modifying treatments are currently unavailable. Therefore, understanding the modifiable risk factors and developing evidence-based interventions for delaying or preventing cognitive impairment is an important challenge. Numerous observational studies have reported a range of potentially modifiable risk factors for dementia, including lower levels of education, midlife hypertension, midlife obesity, diabetes mellitus, smoking, and late-life depression, as well as social isolation, physical inactivity, and hearing loss (2–6). Depression, physical inactivity, and social isolation are particularly important predictors of late-life cognitive impairment (4, 7). Sleep disturbance is also prevalent among older people, representing a risk factor for cognitive impairment (8–11). However, most previous studies have used self-report questionnaires, which can have problems with reliability and consistency due to recall bias or misclassification, particularly among older people, or those with mild cognitive impairment (12–15). Moreover, physical activity questionnaires are not able to capture non-exercise physical activity, which accounts for most total activity energy expenditure among older people and social relationship questionnaires regarding social network size or social engagement cannot accurately measure the duration of contact with family members or friends (16). Therefore, objective measurement of lifestyle components is needed to confirm the relationships between lifestyle factors and cognitive function. Recently, wearable sensors have been used to evaluate lifestyle factors such as physical activity and the sleep-wake cycle in large epidemiological studies (12–15, 17–21). Wearable sensors are non-invasive and cost-effective, and can record total daily movement and the sleep-wake cycle continuously and objectively 24 hours/day without recall bias. In the present study, we developed a wristband sensor enabling quantification of the conversation time for assessing social contact in addition to physical activity and the sleep-wake cycle. Moreover, random forest (RF) regression analysis was conducted to identify risk and protective factors of the lifestyle components associated with Mini-Mental State Examination (MMSE) scores. RF is an ensemble learning method for classification, regression and other functions, which operates by constructing a multitude of decision trees at training time, and outputs the class that is the mode of the classes or mean prediction of the individual trees (22). Machine learning techniques can shorten the time required for big data analysis, and can identify patterns in complex scenarios that are impossible for humans to identify (23). Therefore, machine learning has been applied in disease diagnosis, development of prediction models and identification of risk factors (23–26). The current study aimed to examine the relationship between lifestyle factors collected by wearable sensors and MMSE scores in community-dwelling older people using machine learning.

Abbreviations: BMI, body mass index; CI, confidence interval; MMSE, mini mental state examination; PDP, partial dependency plot; RF, random forest; RMSE, root mean squared error; TST, total sleep time; WASO, wake after sleep onset.

METHODS

Participants

We have been conducting a community-based observation study focusing on lifestyle risk and preventive factors related to dementia in Usuki city, in southern Japan, since 2015. The proportion of the population over 65 years old in Usuki city has reached 38%, compared with 27.3% of the nationwide population in Japan. In the present study, public servants carried out public relations initiatives to recruit participants aged 65 or older without dementia from the entire city using electronic and paper-based media because the lifestyle factors such as physical activity and social isolation are closely related to late-life cognitive impairment (1, 7). From August 2015 to March 2016, a total of 1,020 community-dwelling people agreed to participate in our prospective cohort study examining risk and protective lifestyle factors for dementia among older people. For inclusion, participants met the following criteria: (1) 65 years and older; (2) living in Usuki city; (3) healthy physical and psychological condition; (4) MMSE score 20 points or more and absence of dementia diagnosis or administration of dementia medication; (5) independent function in activities of daily living. The exclusion criteria included a history of other neurological and psychiatric disorders including Parkinson's disease or epilepsy, severe head trauma, alcoholism, severe cardiac failure, and severe hepatic or renal dysfunction, undergoing treatment for cancer, and walking difficulty due to stroke sequelae. All participants underwent a physical examination, evaluation of cognitive function and medical interview at baseline. Height and weight were measured and body mass index (BMI) was calculated as weight in kilograms divided by height in m^2. We collected information about demographic characteristics, including age, gender, education level, and smoking status as well as alcohol consumption and medication history via interviews conducted by trained medical staff at baseline. Moreover, history of chronic disease was defined as a prior diagnosis of stroke, cardiac disease, and hepatic or renal disease as well as cancer. Assessment of

vascular risk factors, such as hypertension, diabetes mellitus, and hypercholesterolemia were based on a detailed clinical history and information of medicine (antihypertensive, antidiabetic, or anticholesterol medication). Because the MMSE is widely used for dementia screening tool, cognitive function was evaluated using the MMSE. The results of MMSE were reviewed by neurologist and clinical psychologist for the primary screening for dementia. Participants were considered to have possible dementia was when they scored <20 points on the MMSE (27). Moreover, we collected the further information regarding dementia diagnosis or administration of dementia medication in the local hospital, and daily living decline due to cognitive impairment from participants and their closest relatives in the face-to-face clinical interview. Diagnosis of dementia was made according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders-5 (28) by a neurologist who used all cognitive and clinical data. Of 1,020 participants, seven participants had other neurological disorders, and one participant had severe renal dysfunction. Four participant had difficulty walking without assistance. Although we recruited participants without dementia via electronic and paper-based media, 13 participants with dementia were identified based on interviews at the first examination. A total of 25 participants with other neurological disorders, severe renal dysfunction, difficulty walking, or dementia were excluded from the present study. The remaining 995 participants were asked to wear a wristband sensor on the wrist for 7–14 days on average every 3 months (total study period 56 days). To eliminate measurement error due to seasonal differences in lifestyle because previous studies using actigraphy measured physical activity and sleep data for a maximum of 3 days (14, 20). Therefore, average annual data were used to examine the relationships between lifestyle factors and cognitive function. A total of 42 participants refused to wear the wristband sensor during the first cycle, and 98 participants had inadequate sensing data for analysis. Thus, the final sample consisted of 855 participants (317 men and 538 women, mean age 73.8 ± 5.8 years, education years 11.8 ± 2.1) with cognitive assessment and valid sensing data (**Figure 1**). The mean age of our participants was rather high, which might reflect the increasing population aging rate in Usuki city. Similarly, previous studies investigated the relationship between sleep or physical activity and cognition in the very elderly people (13, 21). The excluded participants had 1.7 year older (75.5 ± 6.9 years, $p = 0.0097$), slightly lower education years (11.3 ± 1.7, $p = 0.0076$), and lower MMSE scores (median 28, $p = <0.0001$) than 855 participants who were included in our analysis. However, two groups did not differ in the gender distribution (42 men and 98 women, $p = 0.1061$), smoking states (ever smoker 7.4%, $p = 0.1338$), alcohol consumption (ever drinker 38.9%, $p = 0.6168$), and history of chronic diseases (hypertension 55.8%, $p = 0.2651$, diabetes mellitus 13.4%, $p = 0.9861$, hypercholesterolemia 27.3%, $p = 0.2374$, respectively). This prospective study was conducted in accordance with the Declaration of Helsinki, and was approved by the local ethics committee at Oita University Hospital (UMIN000017442). Written informed consent to participate in the study was obtained from all participants.

Wearable Sensor Data

All participants were asked to wear a wristband sensor (Silmee™ W20, TDK Corporation Tokyo, Japan) on the wrist, except while bathing. We excluded data if the heart rate count indicated that the wristband sensor had been removed. Sixteen of 98 participants who had inadequate sensing data (16.3%) did not wear wristband sensor during sufficient period for analysis. Our wearable sensor measured various lifestyle parameters, including heart rate, walking steps, conversation time, total sleep time (TST), sleep efficiency, time awake after sleep onset (WASO), awakening count, and napping time. These parameters were calculated by summing sensing data each day and averaging this over the whole measurement period.

Physical Activity

Physical activity data were detected by a 3-axis accelerometer, which enabled measurement of acceleration in three perpendicular axes. The evaluation circuitry converted the output of a micromechanical acceleration-sensing structure, according to the differential capacitance principle. The accelerometer generated physical activity data regarding walking steps with composite acceleration of 3-axis measurement every time the wearable sensor was moved, and data were captured continuously and summarized in 1 min intervals. Walking steps were identified by capturing frequency bands ranging from 2 to 3 Hz, which synthesized acceleration by the accelerometer. The number of walking steps was calculated by summing the number of steps for each day and dividing this by the number of days of lifestyle data measurement. Therefore, the number of walking steps was represented as the average number of steps per day.

Sleep

Sleep-wake parameters were assessed by the magnitude of synthesized acceleration of the 3-axis accelerometer and cumulative energy. The data were confirmed and adjusted by qualified technicians using visual inspection. Time in bed between bedtime and waking time was determined by the activity count recorded by the wristband sensor. Sleep parameters such as TST, WASO, and sleep efficiency, as well as the awakening count were measured from 18:00 in the evening to 5:59 the following morning (**Figure 2**). Sleep Start was defined as the clock time associated with the beginning of the first 20-min block of sleep without movement (20, 21). TST was defined as the average total number of minutes slept per day. Sleep fragmentation was evaluated by WASO, sleep efficiency, and awakening count. Nocturnal awakening was defined as 20 min of continuous movement from sleep onset to the end of sleep (20, 21). Therefore, WASO and awakening count were calculated by averaging the total number of minutes awake and the number of minutes of sleep per day. Sleep efficiency was calculated as the percentage of TST over the time spent in bed. Although a sleep dairy was not used in this study, the total time in bed between bedtime and getting up was determined by TST and WASO. Nap time was defined as resting without movement on the wearable sensor from 6:00 in the morning to 17:59 in the evening (**Figure 2**).

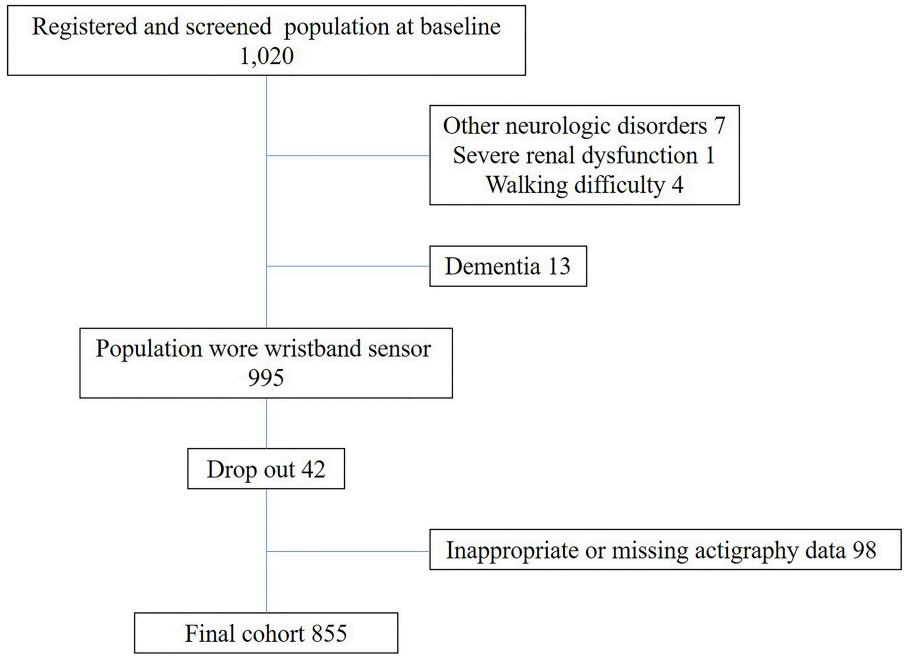

FIGURE 1 | Flow of participant recruitment.

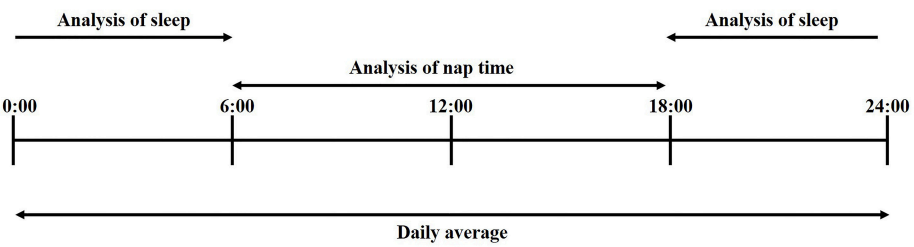

FIGURE 2 | Indices of wearable sensor.

Heart Rate

Heart rate was detected by photoplethysmography. Pulse photoplethysmography is a simple and useful method for monitoring heart rate. Using this method, pulse measurement is based on the irradiation of 573 nm wavelength light, and the conversion of the intensity of reflected light to an electrical signal. The heart rate was calculated by summing pulses per min for each day and dividing this by the number of days in the lifestyle data measurement period. Therefore, heart rate was represented as the average number of pulses per day.

Conversation Time

Our wearable sensor could not detect the content of conversation, but could detect utterances of the participant wearing the wearable sensor, and utterances of nearby people. Sound data were captured continuously during the presence or absence of a conversation every minute. Although the utterances of other individuals were included in the sound data, participating in the conversation was considered to be important for social activity in this study.

Principle of detection

Sound data were collected by a microphone on the wearable sensor, and analyzed to evaluate the conversation time. Our wearable sensor detected sound pressure, which was produced by utterance within a 2-meter radius from the device. The sound pressure range was from 55 to 75 dBA at this distance. The conversation time was defined as the frequency components included in conversation data extracted by signal processing. In detail, the wearable sensor extracted a frequency band corresponding to a human voice from the sound data within the sound pressure range as a sound frame. Conversation was defined as more than four sound frames per minute, during a 1-min period. It is possible that the sound of television viewing or radio listening was detected as conversation due to the detection method based on the sound pressure and

frequency. Therefore, we also quantified the detection rate of television viewing.

Verification of Detection Accuracy

Physical activity

The accuracy of walking step detection was verified by comparing the sensing data and video observation data. Walking steps were simultaneously collected for 9 min by wristband sensor and continuous video monitoring in twenty healthy participants aged 60–80 years (11 men and 9 women). Significant correlation was found between walking steps measured by wristband sensor and those from video observation ($r = 0.9869$, $p < 0.0001$, Pearson correlation, **Supplemental Figure 1**).

Sleep detection

The accuracy of sleep duration detection was verified by comparing the sensing data and video observation data. Sleep duration was simultaneously collected during night time by wristband sensor and continuous video monitoring in five healthy participants aged 20–60 years (5 men). Significant correlation was found between sleep duration from wristband sensor and that from video observation ($r = 0.9995$, $p < 0.0001$, Pearson correlation, **Supplemental Figure 2**).

Conversation time

The accuracy of conversation time detection was verified by comparing the sensing data and self-report data regarding conversation time. Sound data were captured for 50 h in healthy participants aged 30–40 years and analyzed in terms of precision, recall, and F-Measure (**Supplemental Table 1**). The results revealed values of 0.698 for precision, 0.774 for recall, and 0.734 for F-Measure. The false detection rate was calculated to evaluate the false detection of sounds other than conversation, such as television, noise during commuting, or noise during office work. The results of the false detection rate analysis are shown in **Supplemental Table 2**. Furthermore, we verified the false detection rate of sounds, including clothing noise, wind, breath, train, motor vehicles, guitar, piano, violin, cat, dog, bird, vacuum cleaner, tooth brushing, washing machine, dishwasher, and dish-washing, which were likely to be erroneously detected as a conversation. We adjusted each sound to a 55–75 dBA sound pressure range in front of the microphone on the wearable sensor, and input each sound for 100 min continuously to verify the false detection rate. The total false detection rate in the same sound pressure environment was 4.5% (sum of each time)/(number of items *100 min). These results indicate that the conversation time detected by the wearable sensor could be used as a reliable indicator of human conversation time. Moreover, the accuracy of conversation time detection in twenty healthy participants aged 60–80 years was verified by comparing the sensing data and video observation data. Conversation time was simultaneously collected for 9 minutes by wristband sensor and continuous video monitoring in twenty healthy participants aged 60–80 years (11 men and 9 women). Significant correlation was found between conversation time from wristband sensor and that from video observation ($r = 0.8512$, $p < 0.0001$, Pearson correlation, **Supplemental Figure 3**).

Statistical Analysis

RF regression analysis was used to examine the relationships between total daily sensing data and MMSE score. RF is an ensemble learning method that operates by constructing decision trees using bootstrap aggregation, and computes node impurity for every variable. This analysis can be used to rank the variables based on their predictive importance, such as %IncMSE and IncNodePurity. Moreover, confounding factor analysis was performed to build an unadjusted model (model 0), a model adjusted for demographic factors (model 1), and a model adjusted for demographic and vascular risk factors (model 2). The selected variables were used to assess the risk and protective factors for cognitive function using partial dependence plots (PDPs).

RF Regression Analysis

RF was conducted using R (version 3.4.1) and an RF package for Windows 10 (6, 22, 29, 30). The inbuilt bootstrap aggregation procedure of the RF algorithm enables the learning algorithm to be limited to a random sample of features to search. This drastically reduces the variance and avoids the problem of overfitting. The tree was grown on a bootstrap sample ("the bag") by placing two-thirds of the cases in the bag and the remaining one-third "out-of-bag" (OOB) (28). Default values of 500 for the ntree (a hyper parameter to define the number of trees to grow in the model) and p/3 (p indicates the number of predictors) for mtry (a hyper parameter used by the algorithm to determine the count of variables to be randomly sampled for search during each split point) were set. The p/3 value is the default mtry value recommended by the algorithm inventors. The hyper parameters ntree and mtry were not tuned for the variable selection process. Assessment of variable importance was performed using IncNodePurity (a variable importance measure). High IncNodePurity values indicated high importance. A total of 855 samples were used to rank the variables by importance. The selection of top N variables was determined by prediction performance of a model built using 90% of the total 855 samples, assessed by the root mean square error (RMSE) using 10% of the total 855 samples. By applying this rule, the top N variables were selected for which the RMSE value was lowest (**Figure 3**). **Table 1** shows all 17 variables. The adjusted model 1 used 11 variables, including age, gender, education year, and eight wearable sensor variables, whereas the adjusted model 2 used 17 variables for the variable selection process in the RF regression analysis using daily sensing data.

Confounding Factor Analysis

Potential confounding factors included age, gender, education levels, and BMI, alcohol consumption, smoking status, and vascular risk factors, which may affect both lifestyle factors and cognitive function (6). Therefore, the present study used RF regression analysis unadjusted (model 0), adjusted for age, gender, and education year (model 1), and adjusted for all confounding factors (model 2). Multiple linear regression analysis (R version 3.4.1 for Windows 10) was used to identify the effects of confounders and adjust for potentially confounding variables in the model (31). The confounding analysis procedure was conducted in R (30). Multiple linear regression models were

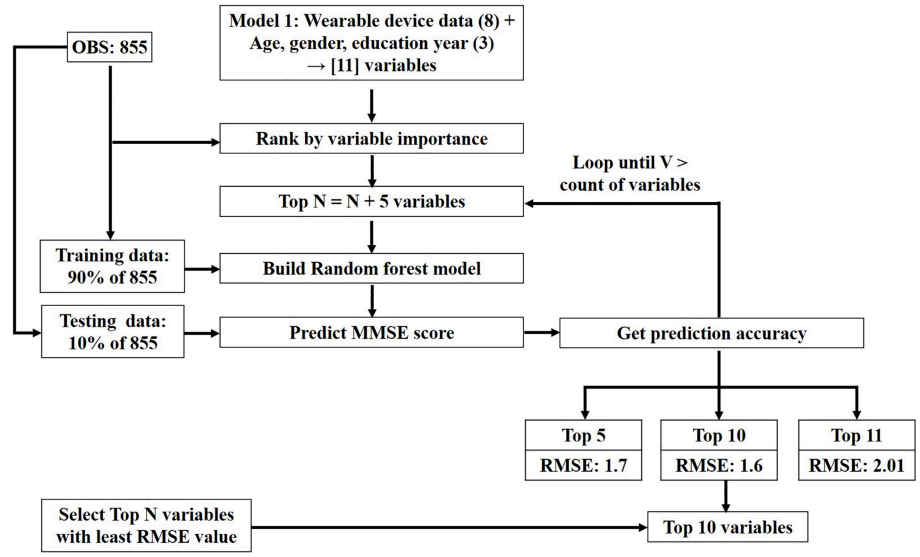

FIGURE 3 | Top N variables selection process.

TABLE 1 | All variables for RF analysis.

Variables
Age (years)
Gender (0; Male, 1; Female)
Education (years)
BMI (kg/m^2)
Smoking status (0; Every day, 1; None, 2; Sometimes)
Alcohol consumption (0; Every day, 1; None, 2; Sometimes)
Hypertension (0; No, 1; Yes)
Diabetes mellitus (0; No, 1; Yes)
Hypercholesterolemia (0; No, 1; Yes)
Walking steps (steps/day)
Conversation time (mins/day)
Heart rate (counts/mins/day)
TST (mins/day)
WASO (mins/day)
Sleep efficiency (%/day)
Awakening time count (counts/day)
Nap time (mins/day)

TST, Total sleep time; WASO, time awake after sleep onset, mins; minutes

TABLE 2 | Confounding factors in model 0, 1, and 2.

Model 0	Model 1	Model 2
Unadjusted	Adjusted for age, gender, education year	Adjusted for age, gender, education year, BMI, hypertension, diabetes mellitus, hypercholesterolemia, alcohol consumption, smoking status

BMI, Body mass index.

built for models 0, 1, and 2 to identify the variables influenced by the confounding factors. The regression coefficients of independent variables were calculated in models 0, 1, and 2 (**Table 2**). The confounding effects on the independent variables were measured by the percentage changes in the estimated regression coefficients as follows: 100 × (adjusted coefficient—unadjusted coefficient)/(unadjusted coefficient). The influenced independent variables were defined as the conditions in which regression coefficient values in model 1 and 2 were increased by more than 200%, decreased by more than 200%, or cases in which the independent variable sign was reversed or independent variable was newly added compared with the model 0. Finally, the independent variables influenced by confounding factors were excluded from the adjusted model 1 and 2.

Risk and Protective Factor Analysis

The variables in model 1 identified by RF and confounding factor analysis were assessed for risk and protective factor analysis. In black box methods like RF analysis, functional relationships between each independent variable and the response variable are assessed using PDP (32). PDP is a simple technique for visualizing partial relationships between the outcome and the predictors. PDP enables visualization of relationships between y and one or more predictors, xj, as detected by RF analysis. In this method, xj is the predictor of interest, X–j represents the other predictors, y is the outcome, and $\hat{}$f(X) is the fitted forest. The partial dependence algorithm functions as follows:

1. For xj, sort the unique values V = {xj}iϵ{1,...,n} resulting in V$*$, where |V$*$| = K. Create K new matrices X(k) = (xj = V$*$k, X–j), $\forall$k = (1,...,K).
2. Drop each of the K new datasets, X(k) down the fitted forest resulting in a predicted value for each observation in all K datasets: $\hat{}$y(k) = $\hat{}$f(X(k)), $\forall$k = (1,...,K).

3. Average the predictions in each of the K datasets, $\hat{y}* k = 1$ nPN $I = 1$ $\hat{y}$ (k) i, $\forall k = (1,...,K)$.
4. Visualize the relationship by plotting V∗ against $\hat{y}*$.

PDPs are generated from the RF model and used to define the variables as risk and protective factors for MMSE scores. The marginal prediction data were extracted from the PDP for all the variables in the RF model. The correlation value was calculated between each independent variable and MMSE score, using the marginal prediction data. Variables with positive correlation values were defined as protective factors, whereas those with negative correlation values were defined as risk factors. Moreover, PDPs for each selected variable were used to determine risk and protective factors.

Contribution and Significance of Risk and Protective Variables

The variables in model 1 identified by RF and confounding factor analysis were applied in multiple linear regression for quantifying the contributions of individual risk and protective variables. $^*P < 0.05$ were considered statistically significant.

Model Prediction Accuracy

Moreover, the model prediction accuracy was verified by linear regression and RF analyses. The training models, including linear regression and RF algorithms, were built using the data set with the variables walking steps, conversation time, TST, heart rate, age, gender, and years of education, and were evaluated with the test data. The performance of the model was evaluated by the prediction RMSE with the lowest value. We used 90% of the 855 samples for training, and 10% of the 855 samples for testing.

RESULTS

Clinical and Demographic Characteristics of Participants and Wristband Sensor Data

Table 3 summarizes the sociodemographic factors, cognitive function, and lifestyle factors of all participants. Participants' mean age was 73.8 years, and 62.9% of participants were female. Lifestyle data were collected from participants for 31.3 ± 7.1 days per year (7.8 days on average every 3 months) using the wristband sensor.

TABLE 3 | Summary of demographic characteristics and wearable sensor data of participants.

CHARACTERISTICS	
Age (years)	73.8 ± 5.8
Gender (M:F)	317:538
Education (years)	11.8 ± 2.1
BMI (kg/m^2)	23.2 ± 3.1
Median MMSE scores	29 (20, 30)
Ever smoker	36 (4.2%)
Ever drinker	354 (41.4%)
PAST HISTORY	
Hypertension	429 (50.2%)
Diabetes mellitus	114 (13.3%)
Hypercholesterolemia	281 (32.9%)
WEARABLE SENSOR DATA	
Walking steps (steps/day)	5452.9 ± 2778.0
Conversation time (mins/day)	219.7 ± 86.3
Heart rate (counts/mins/day)	64.7 ± 6.3
TST (mins/day)	408.4 ± 69.1
WASO (mins/day)	22.1 ± 14.1
Sleep efficiency (%/day)	1.0 ± 0.0
Awakening time count (counts/day)	0.5 ± 0.3
Nap time (mins/day)	48.7 ± 39.3

M, Male; F, Female; BMI, Body mass index; MMSE, Mini-Mental State Examination; TST, Total sleep time; WASO, time awake after sleep onset; (20, 30), the range of MMSE value is from 20 to 30; mins, minutes.

RF Regression Analysis Using Daily Sensing Data

Selecting Important Variables

RF regression analysis using sensing data revealed that the five variables (walking steps, conversation time, TST, and WASO as well as heart rate) in model 0 were selected. Moreover, the top 10 variables (walking steps, conversation time, TST, and WASO, sleep efficiency, awakening time count, naptime, heart rate as well as age and education years) in model 1 were selected based on the lowest RMSE value (1.6).

Confounding Factor Analysis

In model 1, three variables, including sleep efficiency, awakening time count, naptime were newly selected and WASO exhibited a <200% decrease in the estimated parametric values (−440.91%). Therefore, these variables were excluded for next step analysis. Finally, four variables (walking steps, conversation time, TST and heart rate) were included in model 1 for risk and protective factor analysis. The IncNodePurity value of each variable is 313.7 in walking steps, 258.8 in TST, 225.1 in heart rate, and 220.3 in conversation time (**Figure 4**). The variables regarding physical activity were the most important lifestyle factors associated with cognitive function. In model 2, TST exhibited a sign change in the multiple linear regression models. Therefore, the number of walking steps, conversation time, and heart rate remained significant after the RF regression and confounding factor analysis.

Risk and Protective Factor Analysis

Four variables in model 1 were assessed in the protective and risk factor analysis. The number of walking steps, conversation time, and heart rate exhibited positive correlations with MMSE score and were categorized as protective factors for cognitive function, whereas TST was categorized as a risk factor. PDPs of walking steps and heart rate revealed continuously increased MMSE scores. The inclination of the graph, however, began to reverse by the boundary of the specified threshold in the PDP of conversation time and TST (**Figure 5**). The specified threshold was 321.1 min for conversation time and 495.1 min for TST. Therefore, conversation time and TST were not conclusive risk factors, and appeared to become protective or risk factors according to the length of time. An appropriate duration of sleep for preventing cognitive impairment was 291.6–495.1 min,

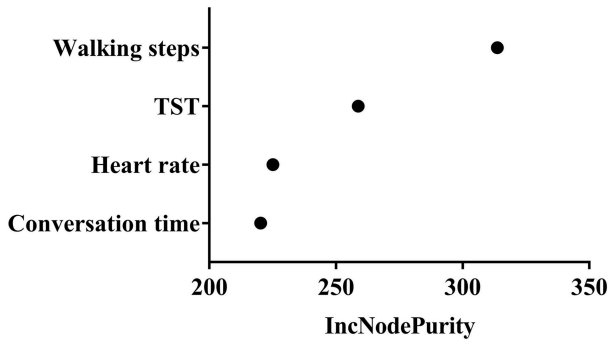

FIGURE 4 | Variable importance measure. The IncNodePurity value of each variable is 313.7 in walking steps, 258.8 in TST, 225.1 in heart rate, and 220.3 in conversation time.

whereas sleep duration of more than 434.1 min exerted a negative effect on cognitive function. Similarly, an appropriate duration of conversation time for preventing cognitive impairment was 80.8–321.1 min, whereas conversation time of more than 321.1 min exerted a negative effect on cognitive function. In addition, the relationship between conversation time and physical activity was investigated to determine why conversation time beyond the specified threshold was identified as a risk factor. Linear regression analysis was performed after transforming the data to the normal distribution. The results revealed that the walking steps was not correlated with conversation time in participants exhibiting <1.125 min (transformed value, mapping value: 320 min) of conversation ($p = 0.181$, **Figure 6**), but was negatively correlated with conversation time in participants exhibiting more than 1.126 min (transformed value, mapping value: 321 min) of conversation ($p = 0.0117$, **Figure 6**).

Contribution and Significance of Risk and Protective Variables

To quantify the individual risk and protective variable contributions, multiple linear regression was performed using only the selected four variables (walking steps, TST, heart rate, and conversation time) after transforming the data to the normal distribution (**Table 4**). The results revealed that walking steps, heart rate, and conversation time were categorized as protective factors for cognitive function (contribution value: 0.4116, 0.1071, and 0.0612, respectively), whereas TST was categorized as a risk factor (contribution value: −0.1128). The walking steps was highly significant.

Model Prediction Accuracy

The prediction accuracy in the RF model was better than that in the linear regression model (**Table 5**).

DISCUSSION

The present study evaluated lifestyle components, including physical activity, sleep, and conversation, as well as heart rate, using a wearable sensor in a large sample of community-dwelling older people, and constructed a machine learning model to predict cognitive impairment. RF regression analysis adjusted for age, gender, and education years identified four significant variables: walking steps, conversation time, TST, and heart rate. Specifically, walking steps, conversation time, and heart rate remained significant after the RF regression analysis was adjusted for demographic and vascular risk factors. Moreover, the walking steps, conversation time, and heart rate were identified as protective factors for cognitive function, whereas TST was categorized as a risk factor for cognitive function. PDPs of walking steps and heart rate revealed continuously increased MMSE scores. TST and conversation time, however, indicated that the tendency in the PDP graph was reversed at the boundary of a specified threshold. Thus, these variables tended to have a protective effect on cognitive function within a particular range of time, whereas longer periods of time exceeding a particular threshold were risk factors for cognitive function. Because RF regression analysis and PDP graph cannot quantify individual variable contribution, multiple linear regression analysis was performed to quantify individual contribution of the selected variables and also to find its statistical significance. Although the multiple linear regression showed that only the number of walking steps was significant, both RF and linear regression analysis revealed similar results regarding the risk and protective factors for cognition. Moreover, the PDP graph exhibited a reversal at the boundary of a specified threshold in TST and conversation time, which was not detected by linear regression. Therefore, we selected four lifestyle variables related to MMSE scores by RF regression analysis and risk and protective factor analysis of each variable were performed using PDPs. The current findings highlight the importance of physical activity, sleep, and conversation in preventing cognitive impairment among community-dwelling older people.

Numerous studies have examined the beneficial effects of physical activity on cognitive function among older people. A meta-analysis of 15 prospective cohort studies reported protective effects of vigorous exercise against cognitive decline (hazard ratio 0.62, 95% CI 0.54–0.70) (33). Another meta-analysis of 16 studies reported a lower risk ratio of dementia (0.72, 95% CI 0.60–0.86) in the highest physical activity group compared with the lowest physical activity group (34). Moreover, several cross-sectional and prospective studies using actigraphy reported that greater daytime movement was protective against cognitive impairment and dementia (12–15). The results of our cross-sectional study were consistent with previous studies regarding the relationship between total daily movement and cognitive function, and suggested that non-exercise physical activity, such as movement around the house and fidgeting is important for delaying cognitive impairment among older people. Several potential mechanisms have been suggested to explain the beneficial effects of physical activity on cognitive function. Physical activity may reduce brain amyloid deposition and increase brain function by decreasing vascular risk factors, including obesity, hypertension, and diabetes (35–37).

Sleep is important for brain plasticity and memory consolidation (38) and sleep disturbance is a common problem for older people as well as patients with mild cognitive

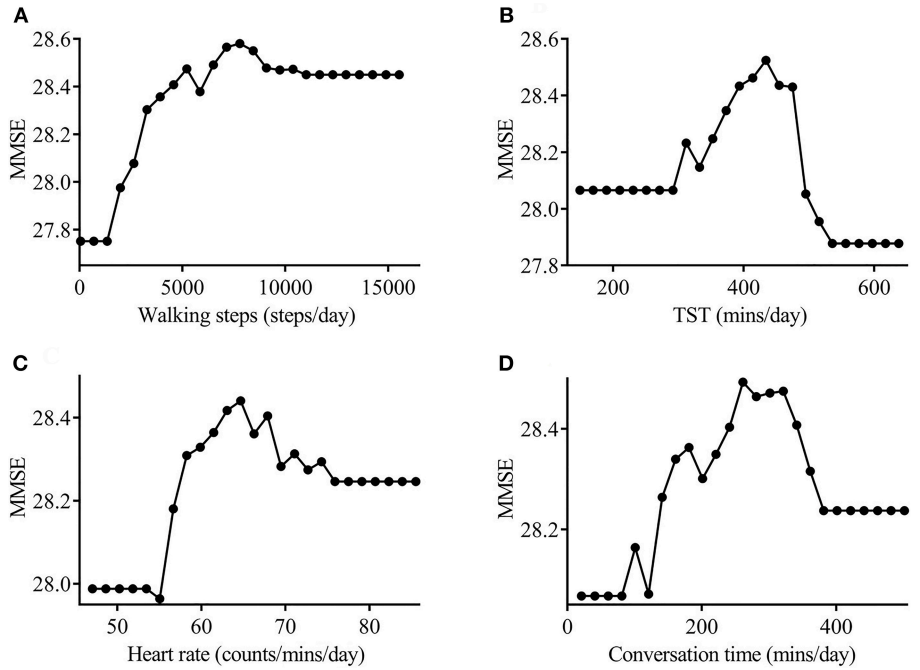

FIGURE 5 | Partial dependency plot for the actinography data. The number of walking steps **(A)**, heart rate **(B)**, and conversation time **(C)** showed a positive correlation with MMSE score and were categorized as protective factors for cognitive function (correlation values: 0.71, 0.547, and 0.396, respectively). TST **(D)** showed a negative correlation with MMSE scores, and was categorized as a risk factor for cognitive function (correlation value; −0.245). The inclination of the graph began to reverse by the boundary of specified threshold in the PDP of conversation time and TST (321.1 min and 495.1 min, respectively). MMSE, Mini-Mental State Examination; TST, tonal sleep time.

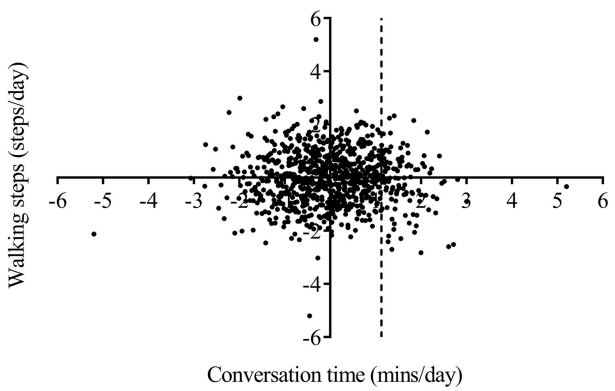

FIGURE 6 | Correlation analysis between the number of walking steps and conversation time. The daily number of walking steps was not correlated with conversation time in participants exhibiting <1.125 min (transformed value, mapping value: 320 min) of conversation, and decreased with increasing conversation time in participants exhibiting more than 1.126 min (transformed value, mapping value: 321 min) of conversation.

TABLE 4 | Risk and protective variables contribution.

	Estimate	SE	*t*-value	*P*-value
Walking steps (steps/day)	0.4116	0.0722	5.702	1.63e−08*
TST (mins/day)	−0.1128	0.0731	−1.542	0.123
Heart rate (counts/mins/day)	0.1071	0.0726	1.476	0.140
Conversation time (mins/day)	0.0612	0.0721	0.849	0.396

*SE, Standard error; TST, Total sleep time; mins, minutes *P < 0.05.*

TABLE 5 | Model accuracy.

	Training model			Prediction model		
	R^2 value	MSE	RMSE	R^2 value	MSE	RMSE
Linear regression	0.119	3.155	1.776	0.175	2.955	1.719
RF regression	0.775	0.796	0.892	0.759	0.863	0.929

RF, Random forest; MSE, Mean Squared Error; RMSE, Root mean squared error.

impairment and dementia (8–10, 39). Several cross-sectional or prospective studies reported that shorter and longer sleep duration may be important risk factors for subsequent cognitive impairment (8, 10, 19). The current results indicated that an appropriate duration of sleep was important for delaying cognitive impairment, whereas longer sleep duration (more than 434.1 min) exerted a negative effect on cognitive function in older people. One prospective study of the relationship between sleep duration and the risk of dementia reported that the risk of dementia was increased among individuals with particularly

long sleep durations (8 and more than 9 h), compared with those with normal sleep durations (6 and 7 h). These results suggested that longer sleep duration might be a risk factor for cognitive impairment among older people. The sleep-wake cycle is associated with the clearance of brain amyloid-β protein (40), while shorter sleep duration was associated with greater brain amyloid burden on amyloid positron emission tomography (41). However, the mechanisms underlying the relationship between longer sleep duration and dementia remain unclear. Longer sleep duration may increase the risk of dementia, function as an early symptom of dementia, or be associated with sleep disorder-related breathing and smoking habits (10).

The present findings identified daily heart rate as a protective factor for cognitive function among community-dwelling older people. To our knowledge, no previous reports have examined the relationship between heart rate and cognitive function. A previous study reported relationships between resting heart rate, depression, and cognitive impairment in patients with ischemic stroke, and relationships between reduced heart rate variability and cognitive impairment among older women (42–44). Further studies are needed to confirm the influence of heart rate on cognitive function among older people.

Importantly, the current results revealed that conversation time was an important predictive factor for MMSE score. Social isolation and subjective loneliness are increasingly recognized as risk factors for cognitive impairment and dementia among older people (7, 16). A meta-analysis of social activity reported that the risk of developing dementia was increased in individuals with less social participation (relative risk 1.41, 95% CI 1.13–1.75) and less social contact (relative risk 1.57, 95% CI 1.32–1.85) (16). An intervention study reported that active social engagement, including contact with family and friends and positive social support and engagement in leisure activities have beneficial effects for preventing cognitive impairment and dementia (45). In present study, we quantified communication by detecting participants' utterances, using conversation time as a surrogate parameter of social isolation. Few previous studies have examined the relationship between conversation time and cognitive function. Although an appropriate duration of conversation time tended to have a protective effect on cognitive function, we found that longer durations of conversation time (more than 321.1 min) exerted a negative effect on cognitive function among older people. One possible explanation is related to the different effects of conversation time on cognitive function according to the length of time, because longer conversation time was associated with a decreased number of walking steps. Therefore, our results suggest the importance of balance between the duration of conversation and the duration of physical activity. The current results were consistent with previous studies regarding the relationship between communication and cognitive function, highlighting the importance of spending an appropriate proportion of time engaging in conversation. The mechanisms underlying social activity and cognition support the cognitive-reserve hypothesis, which suggests that participation in intellectual, social and physical activities stimulates brain function, resulting in the prevention of dementia (46). In animal studies, mice raised in an enriched environment have been reported to exhibit greater neurogenesis and increased synaptic density, and amyloid precursor protein transgenic mice have been found to exhibit decreased brain amyloid-β deposition in enriched environments (47, 48). Another potential mechanism is that social contact or social support may lead to decreased stress and increased motivation to perform health-related behaviors, resulting in the prevention of dementia (7).

The present study has several limitations that should be considered. First, the study could not determine the causal direction of the association between lifestyle factors and cognitive function because of its cross-sectional design. Second, we were unable to exclude factors influencing cognitive reserve, such as past, or current occupation and engagement in cognitive and social activity, which may affect lifestyle and cognitive function. Third, cognitive function was evaluated only by MMSE and information regarding depression was not collected. The MMSE is a very crude measure of cognition and questionable accuracy for detecting dementia. Although we collected the clinical information to define the present or absence of dementia, the patients with possible dementia could not be excluded completely from participating in the current study. Therefore, further studies assessing a broader range of cognitive domains should be needed to confirm our results. Forth, it is possible that the sound of television viewing or radio listening was detected as conversation due to the detection method based on sound pressure and frequency. Therefore, further studies are needed to improve the reliability of our sensing data. Conversation time may have included sleep or nap time during television viewing or radio listening. The relationship between conversation time and sleep or nap time, however, suggested that the possibility of sleeping being included in the daily conversation time was only 6.4%, which would not be expected to influence the results.

In conclusion, the current study revealed that lifestyle factors such as physical activity, sleep, and social activity were associated with global cognitive function among older people. Physical activity and heart rate were positively associated with cognitive function. Moreover, an appropriate balance between the durations of sleep and conversation appears to be important for cognitive function. These results may contribute to the development of new evidence-based interventions for preventing cognitive impairment and improving health and wellbeing among older people.

ETHICS STATEMENT

This prospective study was conducted in accordance with the Declaration of Helsinki, and was approved by the local ethics committee at Oita University Hospital (UMIN000017442).

AUTHOR CONTRIBUTIONS

NK and EM conceived and designed the trial. SN and MK developed the wearable sensor. YA, KY, MI, DH, YS, AN, SU, HF, SI, and TK performed the PET study and conducted the data analysis. AE performed the neuropsychological

assessment. KS and MJ helped with the recruitment of participants.

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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.2019.00401/full#supplementary-material

Supplemental Figure 1 | Linear correlation analysis of walking step. Significant correlation was found between walking steps from wristband sensor and those from video observation ($r = 0.9869$, $p < 0.0001$, Pearson correlation).

Supplemental Figure 2 | Linear correlation analysis of sleep duration. Significant correlation was found between sleep duration from wristband sensor and that from video observation ($r = 0.9995$, $p < 0.0001$, Pearson correlation).

Supplemental Figure 3 | Linear correlation analysis of conversation time. Significant correlation was found between conversation time from wristband sensor and that from video observation ($r = 0.8512$, $p < 0.0001$, Pearson correlation).

Supplemental Table 1 | Results of conversation detection.

Supplemental Table 2 | Analysis of false detection rate.

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Chronic Traumatic Encephalopathy: A Brief Overview

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Chronic Traumatic Encephalopathy (CTE) is a debilitating neurodegenerative disease, which has been increasingly reported in athletes, especially American football players, as well as military veterans in combat settings, commonly as a result of repetitive mild traumatic brain injuries (TBIs). CTE has a unique neuropathological signature comprised of accumulation of phosphorylated tau (p-tau) in sulci and peri-vascular regions, microgliosis, and astrocytosis. As per most recent disease classification, the disease manifests itself in four different stages, characterized by widespread tauopathy. Clinically, CTE has a more subtle presentation, as patients often present with two distinct phenotypes, with one subtype initially presenting with affective changes, and the other subtype with more cognitive impairment. On a genetic basis, there are no clear risk factor genes. Although ApoE4 carriers have been reported to suffer more severe outcome post TBI. As there are no disease modifying regimen for CTE, the newly developed TBI treatments, if administered in a time sensitive manner, can offer a potential viable option. Prevention is another key strategy that needs to be implemented in various sports and military settings. Providing education for safe practice techniques, such as safe tackling and hitting, and providing ready access to full neuropsychiatric assessment by team physician could have measurable benefits. The combination of advanced of research techniques including neuroimaging, as well as increasing public awareness of CTE, offers promising vistas for research advancement.

Keywords: TBI, CTE, tau & phospho-tau protein, concussion and sports, dementia pugilistica

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Chronic Traumatic Encephalopathy (CTE) is a distinctive tau-protein associated neurodegenerative disease. There has been a rise of CTE diagnosis in athletes, especially American football players, as well as in military veterans in combat settings (1, 2). Although CTE has been publicly recognized relatively recently, it was first described as "punch drunk" syndrome in a classic article by Martland et al. (3). The report was focused on a number of boxers who had suffered repetitive head blows throughout their careers, and were presenting with both psychiatric symptoms as well as severe memory and neurocognitive deficits, analogous to typical dementia patients (3). The disease nomenclature evolved into "dementia pugilistica" (4), and finally CTE in 1949 (5).

CTE has a unique neuropathological characteristic, comprised of accumulation of phosphorylated tau (p-tau) in sulci and peri-vascular regions, microgliosis, and astrocytosis. These pathological changes lead to progressive debilitating neurodegeneration. Based on the pattern of pathological progression, CTE is divided into four respective stages (**Figure 1**). In stage I CTE, the brain grossly appears normal, but p-tau is found in a finite number of loci, often in the lateral and frontal cortices, as well as proximal to small blood vessels in the depth of sulci. There

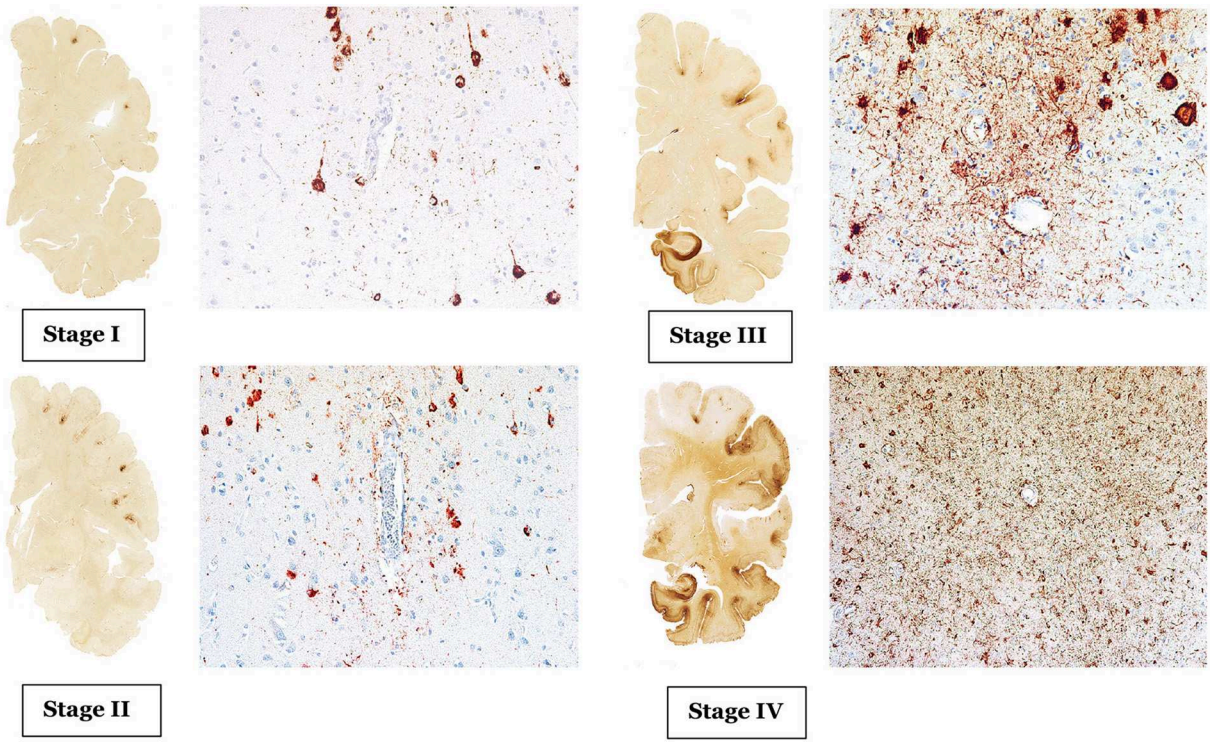

FIGURE 1 | The above images are depiction of the McKee's four stages of CTE [adopted from Mez et al. (6), Figures 1, 2].

might be a scant number of neurofibrillary tangles (NFTs) and neurites in the locus coeruleus. In stage II, localized macroscopic abnormalities might be noted. On gross anatomical sections and neuroimaging, enlargement of lateral ventricles, cavum septum pellucidum with or without fenestration, as well as pallor of the locus coeruleus and substantia nigra are observed. There are multiple foci of p-tau within the depth of sulci, and there is an emergent spreading pattern. In stage III, most gross pathological sections show macroscopic abnormalities. There is global brain weight loss, mild frontal lobe and temporal lobe atrophy, and dilation of the ventricles. One half of CTE patients display septal abnormalities, including cavum septum pellucidum. P-tau pathology spreads, involving the frontal, temporal, parietal and insular cortices. In stage IV, the reduction in brain weight is dramatic, and brain weights of 1,000 g (compared to 1,300–1,400 g in normal brains) have been reported. There is profound atrophy of the frontal, medial temporal lobes, as well as anterior thalami. There is also atrophy of the white matter tracts. The majority of stage four patients have septal abnormalities. The spread of the p-tau affects most regions, including the calcarine cortex (7, 8). Abnormalities in phosphorylated 43 kDa TAR DNA binding protein (TDP-43) is also seen in most CTE patients. The parenchymal TDP-43 pathology is also progressive in nature similar to the anatomical pattern of spread of p-tau. TDP-43 immunoreactivity is found in almost all cases of stage IV disease (7).

The CTE clinical phenotype is yet to be clearly defined. The following paragraphs outline attempts of characterization of CTE symptoms in the various stages of the disease process (**Table 1**). According to McKee's classification, in stage I, a typical CTE patient is asymptomatic, or may complain of mild short term memory deficits and depressive symptoms. Mild aggression may be observed. In Stage II, the mood and behavioral symptoms could include behavioral outbursts and more severe depressive symptoms. In Stage III, patients typically present with more cognitive deficits, including memory loss, executive functioning deficits, visuospatial dysfunction, and apathy. In Stage IV, patients present with advanced language deficits, psychotic symptoms including paranoia, motor deficits, and parkinsonism.

Jordan et al. (10) were one of the first to clinically characterize the disease. They divided CTE clinical presentations into three domains: behavioral/psychiatric, cognitive, and motor. The behavioral and psychiatric domain included aggression, depression, apathy, impulsivity, delusions including paranoia, and suicidality. The cognitive domain included diminished attention and concentration, memory deficits, executive functioning deficits, visuospatial dysfunction, language deficits, and dementia. Finally, the motor features consisted of dysarthria, gait abnormalities, ataxia and incoordination, spasticity, and parkinsonism features such as tremors. Based on these clinical features, as well as existent neuropathological information, four diagnostic subtypes were defined, namely "Definite," Probable," "Possible," and "Improbable" CTE.

Stern et al. (11), and related case reports (14, 15), differed in their description of a typical CTE patient, conceptualizing the clinical presentation into two distinct subtypes. The first

TABLE 1 | CTE proposed clinical classifications.

Classification	Diagnostic subgroups	Definition
McKee et al. (9)	Stage I	• Asymptomatic, or mild memory and depressive symptoms.
	Stage II	• Symptoms include behavioral outbursts and severe depression
	Stage III	• Cognitive deficits including memory loss and executive dysfunction
	Stage IV	• Advance language deficits, psychotic symptoms, profound cognitive deficits, and motor features.
Jordan et al. (10)	Definite CTE	• Clinical CTE symptoms with supportive neuropathology
	Probable CTE	• Two or more CTE clinical symptoms consistent with CTE
	Possible CTE	• Consistent with CTE or other neurodegenerative diagnosis such as AD, PD, ALS
	Improbable CTE	• Not consistent with CTE symptoms examples include MS, or brain tumors
Stern et al. (11)	Behavioral CTE subgroup	• Initial presentation of mainly mood/behavioral symptoms
	Cognitive CTE subgroup	• Initial presentation of mainly cognitive impairment
Gardner et al. (12)	Classic CTE	• Initial presentation typically includes parkinsonism with later progression to cognitive symptoms
	Modern CTE	• Early clinical symptoms include mood/affective symptoms with later progression to cognitive symptoms
Montenigro et al. (13)	Traumatic encephalopathy syndrome (TES) I. TES behavioral/mood variant II. TES cognitive variant III. TES mixed variant IV. TES dementia	• Based on core clinical features including cognitive, behavioral, and mood domains • Supportive features including impulsivity, anxiety, apathy, paranoia, suicidality, headache, motor signs, documented functional decline, and delayed onset of symptoms for at least 2 years after significant head impact exposure
	Additional CTE based classification: I. Probable CTE II. Possible CTE III. Unlikely CTE *CTE Biomarkers: (1) Cavum septum pellucidum (2) Normal amyloid-beta CFS level, as opposed to CSF amyloid beta elevation in AD (3) Elevated p-tau/total tau ratio compared to age-matched controls (4) Positive Tau neuroimaging such as Tau-PET imaging (5) Negative amyloid imaging, such as amyloid PET Brain scan, in order to delineate from possible AD (6) Cortical thinning (7) Cortical atrophy	• Probable CTE group has at least one positive CTE biomarker such Tau PET imaging vs. possible have progressive CTE course with any biomarker testing vs. in the unlikely CTE group, TES diagnosis not satisfied or negative Tau imaging or both • If the clinical presentation also included motor signs such as parkinsonism, the modifier "with motor features" was also added

Summary of clinical stages of CTE according to various proposed classifications.

subtype displayed mainly behavioral and mood changes, and the other presented with mainly cognitive impairment. The vast majority of the mood/behavior subtype developed cognitive deficits as the disease progressed. However, relatively few patients of the cognitive group displayed mood or behavior alterations during the course of their illness. In study by Stern et al. (11), the cognitive group patients had a significantly higher probability of developing dementia. They also were significantly older at the time of diagnosis compared to the mood/behavior group patients. The behavioral subgroup of CTE patients can resemble patients suffering from behavioral variant frontotemporal dementia (bvFTD), which makes the clinical diagnosis more challenging. However, typical bvFTD characteristic behavioral manifestations such as apathy and disinhibition are often not seen in CTE patients (11, 16). Given the inherent heterogeneity of bvFTD, as well as similar tauopathic nature of both diseases, distinguishing bvFTD and CTE poses a diagnostic challenge.

Out of the behavioral symptoms of CTE, the association between suicide and CTE remains a topic under scrutiny in the literature. Earlier studies, such as the series of five professional athletes with a confirmed diagnosis of CTE reported by Omalu et al. (17), had suggested a strong relationship between CTE and suicide. The authors further suggested that the etiology of suicidal/parasuicidal behavior in the CTE population might be partly due to tauopathy in the form of neurofibrillary tangles and neuritic threads in strategic limbic brain nuclei such as locus ceruleus. Maroon et al. (18), reviewed 153 pathologically confirmed cases of CTE published between 1954 and 2013. They reported the suicide prevalence in the CTE population and accidental deaths to be 11.7 and 17.5%, significantly higher than the general population levels of 1.5 and 4.8%, respectively (18). Proponents of opposing view suggest that suicides have been mostly reported in earlier stages of CTE, and the association between disease progression and suicide remains unclear at this time (19).

In a meta-analysis of 158 case studies by Gardner et al. (12), CTE clinical symptoms were divided into "classic" vs. "modern" CTE symptoms, to draw a distinction between an older description of CTE cases centered mostly on boxers compared to a more evolved clinical description which also applies to professional American football players. Whereas, the "classic" CTE symptoms typically included dysarthria, movement difficulties, and later progression to memory deficits, the "modern" CTE picture also included neuropsychiatric symptoms, such as depressive symptoms, paranoia, social withdrawal and isolation, compromised judgment and aggression. Cognitive deficits such as memory decline, executive dysfunction, language, and information processing deficits emerge later in the course of the disease process (12).

Since the definition of CTE primarily depends on pathological characteristics, there is a proposed alternative clinical term of traumatic encephalopathy syndrome (TES) by Montenigro et al. (13), describing the clinical sequelae of repetitive TBIs. The authors based this classification on a review of 202 published cases. TES is a more encompassing diagnosis and can be subdivided into four subcategories, including TES behavioral/mood variant, TES cognitive variant, TES mixed variant, and TES dementia. The proposed TES diagnosis was based on the existence of five general criterion, three core clinical features, and nine supportive features. Using existent biomarkers* (**Table 1**), additional diagnostic qualifiers were proposed, which included "Probable," "Possible," and "Unlikely" CTE (9, 13). The proposed TES diagnosis also contained temporal qualifiers and included "progressive course," "stable course," and "unknown/inconsistent course." If the clinical presentation also included motor signs such as parkinsonism, the modifier "with motor features" was also added.

As our understanding of CTE grows, there are a number of challenges and critiques that need to be addressed. One hypothesis as an alternative to the phenomena CTE, is a diminished "cognitive reserve" theory. The theory states that repetitive neurotrauma leads to a reduction in cognitive reserve and acceleration of development of an underlying neurodegenerative disorders (20, 21). If this theory held true, it would imply that CTE and AD are on the same neuropathological spectrum. This assertion deserves further analysis. Similar to AD, the Tau isoforms in CTE also consist of the mix of three-repeat (3R) and four-repeat (4R) isoforms. However, according to a recent report by Falcon et al. (22), the tau filaments extracted from the brains of CTE patients also contain a unique ß-helix region with a hydrophobic cavity, which is not present in the brains of AD patients. The cavity contains an additional cofactor that is thought to play a functional role in tau propagation. Falcon et al. (22) suggest that the location of tau inclusions in proximity to blood vessels, suggest that cofactors necessary for tau assembly may cross the blood brain barrier after head trauma. The authors further argue that the fact that brain trauma leads to CTE in only a subgroup of injured population, might be related to higher level of cofactors in the more susceptible individuals. These cofactors might provide a therapeutic target for prevention of tau assembly and development of CTE post injury (22).

An alternative theory proposes that the psychiatric symptoms such as depression and anger reported in CTE patients are independent of the CTE disease process and are reported in a cofounded fashion. The proponents of this hypothesis have cited prior studies such as the one reported by Weir et al. (23), in which 1,063 former NFL players were asked whether they have experienced bouts of anger. It was reported that 30.7% of the players ages 30–49, and 29.3% of the players ages 50 or above reported bouts of anger. However, the authors also noted that the reported measures of anger was indeed lower than the one reported for the general US population, which was 54.8% for men between 30 and 49, and 47.2% for men above the age of 50 (23). Though the arguments pertaining to comorbidity of psychiatric symptoms and neurodegenerative diseases such as CTE, are difficult to verify based on neuroimaging and neuropathological findings, one can apply similar arguments to psychiatric symptoms of any neurodegenerative condition such as AD, bvFTD, Parkinson Disease (PD) or amyotrophic lateral sclerosis (ALS).

Another important source of diagnostic confusion is the clinical delineation between CTE and prolonged post-concussive syndrome (PCS), especially given prior reports indicating that ∼10–20% of individuals who suffer concussions, experience prolonged symptoms. Chronic Postconcussive Syndrome (CPCS) refers to persistence of PCS symptoms leading to impaired functional and often athletic performance lasting longer than 1 year. CPCS symptoms include headache, dizziness, impaired attention, memory and executive functioning deficits, depression and irritability symptoms (10). King and Kirkwilliam coined the term, "Permanent PCS" to refer to those with PCS symptoms persisting an average of 6.9 years after the initial concussion. Furthermore, they reported that a significant number of permanent PCS patients (40–59%) also had premorbid or postmorbid neuropsychiatric conditions such as depression, anxiety, PTSD, and/or pain (24). As argued by Jordan et al. (10), CPCS is clinically distinguishable from CTE, based on its temporal relationship to the acute concussive event. A thorough and accurate temporal history remains key in the neurological assessment. Furthermore, headache is a central feature of CPCS but not commonly reported in CTE. Although arguable, McKee stages I and II patients could present with headaches, further adding the complexity of possible overlap of CTE & CPCS (9). The CPCS diagnosis remains controversial, as it is not clear whether it is tauopathic in nature. Hence, the dividing lines of CPCS and McKee's stages I and II clinical characteristics are not fully solidified.

Clear genetic predispositions to CTE have not been reported. However, the ApoE4 gene, the most well-known risk factor for Alzheimer's Disease (25), has been associated with greater cognitive deficits and a more protracted recovery period after Traumatic Brain Injury (TBI) (11). A study on a group of boxers has reported more severe outcomes in individuals carrying at least one ApoE4 allele (26). Conversely, ApoE3 might confer neuroprotection, even in the presence of a progressive CTE pathology (15). Another proposed protective factor associated with more favorable post TBI recovery is cognitive reserve, as measured by premorbid IQ and total

intracranial volume (27). Other genetic candidates for further study include the microtubule-associated protein tau (MAPT) gene, the progranulin (GRN) gene, and the chromosome nine open reading frame 72 (C9ORF72) gene (11).

The pathologic synergism of tauopathy and neuroinflammation is increasingly being recognized. Extracellular secretion of hyperphosphorylated tau is thought to activate microglia and astrocytes, leading to production of pro-inflammatory cytokines such as IL1ß, and TNFa, in turn leading to activation of tau kinases such as p38 and cdk5, and further tau phosphorylation. This process creates a vicious perpetual tauopathy and neuroinflammaion cycle (28). Given the robust association between repetitive traumatic brain injuries and risk of CTE (1), timely treatment of TBI could diminish the development of CTE. The pro-inflammatory nature of TBI has been previously reported (13), and anti-inflammatory agents such as minocycline with N-Acetylcysteine, a potent anti-oxidant, administered in acute to subacute time windows post TBI, offer a promising therapeutic regimen (29, 30). The development of a time sensitive protocol, resembling the treatment algorithm for ischemic stroke, would potentially measure long term outcome in post TBI recovery and prevention of development of subsequent CTE pathology (29).

There are currently no disease modifying medications for CTE, making prevention the most effective way of combating this debilitating neurodegenerative disease (31). Given the frequency of head collisions in contact sports such as American football, prevention of head trauma will require a cultural shift in the way the sport is taught and practiced. Training for safe practice techniques, such as safe tackling and hitting, while penalizing reckless hits will offer measurable benefits. Further changes must include creating an environment of safety, in which players are encouraged to report symptoms to referees, coaches as well as to team physicians. Furthermore, establishing a baseline neurocognitive profile could be used as a clinical reference marker to track changes in players' neuropsychiatric presentation. It is incumbent upon the team physicians to remove players from the field who have suffered even a mild uncomplicated TBI for further assessment (32).

There are a number of existent CTE related challenges to address. Although the incidence of sport related concussion has been reported to range from 1.6 million to 3.8 million, the incidence and prevalence of CTE remains largely unknown (33). One explanation for this lapse of knowledge is perhaps due to the fact that athletes exposed to cumulative subconcussive hits, which exert sufficient force to confer neuronal damage but initially have no overt clinical symptoms, are often not assessed or diagnosed in a timely manner (34). Large scale prospective studies, such as tracking athletes with multiple TBIs over a predefined period, would add to our understanding of the natural course and phenomenology of the disease. CTE is increasingly reaching the public spotlight via the mass media. Continuous efforts to diagnose, assess, and treat this devastating illness are needed. The exponential advancement in neuroimaging techniques and understanding of the neuropathological mechanisms of the illness will lead to earlier diagnosis and timely treatment interventions.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Altered Time Awareness in Dementia

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Our awareness of time, specifically of longer intervals spanning hours, days, months, and years, is critical for ensuring our sense of self-continuity. Disrupted time awareness over such intervals is a clinical feature in a number of frontotemporal dementia syndromes and Alzheimer's disease, but has not been studied and compared systematically in these diseases. We used a semi-structured caregiver survey to capture time-related behavioral alterations in 71 patients representing all major sporadic and genetic syndromes of frontotemporal dementia, in comparison to 28 patients with typical Alzheimer's disease and nine with logopenic aphasia, and 32 healthy older individuals. Survey items pertained to apparent difficulties ordering past personal events or estimating time intervals between events, temporal rigidity and clockwatching, and propensity to relive past events. We used a logistic regression model including diagnosis, age, gender, and disease severity as regressors to compare the proportions of individuals exhibiting each temporal awareness symptom between diagnostic groups. Gray matter associations of altered time awareness were assessed using voxel-based morphometry. All patient groups were significantly more prone to exhibit temporal awareness symptoms than healthy older individuals. Clinical syndromic signatures were identified. While patients with typical and logopenic Alzheimer's disease most frequently exhibited disturbed event ordering or interval estimation, patients with semantic dementia were most prone to temporal rigidity and clockwatching and those with behavioral variant frontotemporal dementia commonly exhibited all these temporal symptoms as well as a propensity to relive past events. On voxel-based morphometry, the tendency to relive past events was associated with relative preservation of a distributed left-sided temporo-parietal gray matter network including hippocampus. These findings reveal a rich and complex picture of disturbed temporal awareness in major dementia syndromes, with stratification of frontotemporal dementia syndromes from Alzheimer's disease. This is the first study to assess symptoms of altered temporal awareness across frontotemporal dementia syndromes and provides a motivation for future work directed to the development of validated clinical questionnaires, analysis of underlying neurobiological mechanisms and design of interventions.

Keywords: time perception, clockwatching, Alzheimer's disease, frontotemporal dementia, primary progressive aphasia, semantic dementia, voxel-based morphometry

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INTRODUCTION

Our capacity to experience and calibrate the passage of time anchors us in the flux of sensory experience and allows us to track external events, conduct our daily affairs and most fundamentally, maintain a sense of self-continuity from past to future (1–3). Our awareness of time is not dictated simply by the clock: it is a complex and elastic, subjective psychological construct, encompassing multiple, hierarchically embedded scales, ranging from fractions of a second to an entire lifetime (4–6). Brief time intervals are more directly accessible to laboratory analysis and accordingly, most neuropsychological data on time perception and awareness relate to shorter timescales (7–9). However, many of our psychologically salient experiences unfold over longer timespans that are challenging to study experimentally.

Distributed cortical and subcortical brain networks—including prefrontal and insular cortices (2, 5, 10–14), parietal cortex (15–17), hippocampus (3, 18–20), and basal ganglia (21–23)—have been associated with temporal encoding at different time scales and mediating different kinds of temporal computations (4, 6, 24, 25). Normal temporal awareness across timescales and in particular, integration of external clock time with internal bodily or "subjective" time is likely to depend on interactions between large-scale neural networks: for example, fronto-striatal time-keeping circuitry (26–29) operating in concert with the so-called "default mode" temporo-parietal network that mediates self-awareness and self-projection (12, 25, 30).

This neural network paradigm provides a rationale for anticipating and understanding alterations of subjective temporal awareness accompanying neurodegenerative pathologies. These pathologies characteristically and selectively target the distributed neural networks implicated in temporal processing in the healthy brain (31–35). It follows that neurodegenerative disorders are likely to have overlapping but separable phenotypes of abnormal temporal awareness, arising from the profiles of network involvement they produce. From a neurobiological perspective, better definition of temporal processing mechanisms in these diseases might provide novel insights into their pathophysiology. However, the clinical features and brain substrates of altered temporal awareness in major dementias such as Alzheimer's disease (AD) and in particular, frontotemporal dementia (FTD), have not been systematically assessed and compared.

Several questionnaires and interview protocols have been developed to look at subjective time awareness in both healthy and clinical populations (36–39). Notably, the Autobiographical Interview (40) has been useful in demonstrating impaired retrieval of past autobiographical memories (41, 42), and diminished ability to project into the future (43) in patients diagnosed with AD. Distortions of subjective time estimation (44–46), confusion about temporal ordering and reduced self-projection in time (46–49) have also been described in AD. Deficits of temporal processing in AD are not simply due to deteriorating episodic memory for the details of events, but rather the sequencing of those events in relation to one another, suggesting a more fundamental disorder of the mental timeline.

Temporal processing abnormalities have also been observed in FTD. This is a clinically and pathologically heterogeneous group of diseases (31), comprising three canonical clinico-anatomical syndromes: behavioral variant FTD (bvFTD; led by impaired socio-emotional signal processing and reactivity, with atrophy predominantly of prefrontal and insular cortices and their connections) and the language-led syndromes of semantic dementia (SD; led by degradation of vocabulary and conceptual knowledge with selective left anterior temporal lobe atrophy) and progressive non-fluent aphasia (PNFA; led by speech and language output failure with predominant left-sided peri-Sylvian atrophy). Impaired perception and generation of temporal intervals and patterns over short time intervals attributed to a deranged internal "clock" mechanism have been described in bvFTD and PNFA (50, 51). Over longer timescales, difficulties with prospection and retrospection have been documented in bvFTD and SD (49, 52–56). There are currently no validated instruments to assess clockwatching and more general inflexibility or obsessionality around time, although these are frequently associated with both bvFTD and SD (31, 57, 58). Impaired awareness of time and associated disruptions of socio-emotional behaviors in these diseases potentially take a substantial toll on patient well-being and care burden and therefore constitute a significant clinical issue.

Here we addressed the issue of temporal awareness in FTD in a cohort of patients representing all canonical syndromes of FTD (bvFTD, SD, and PNFA), in relation to healthy older individuals and a cohort of patients with typical amnestic AD and its major, language-led variant phenotype, logopenic aphasia (LPA). We surveyed temporal behavioral alterations which we hypothesized on clinical grounds to be particularly pertinent to the target syndromes and to the timeframes of daily life and autobiographical experience. We did not set out in this first study to characterize temporal symptoms in detail. Rather, our principal objective here was to survey the kinds of altered temporal awareness that occur in FTD, to estimate the relative proportions of patients with different FTD syndromes exhibiting symptoms of altered temporal awareness and to explore possible differences with respect to typical and amnestic AD. We assessed structural neuroanatomical correlates of altered temporal awareness using voxel-based morphometry (VBM) of patients' brain magnetic resonance images.

Based on clinical observations, we hypothesized that patients with AD syndromes (both the typical amnestic and the language-led phenotypes) would exhibit particularly prominent disturbances of temporal interval estimation and event ordering. In contrast, we hypothesized that patients with FTD syndromes (especially bvFTD and SD) would particularly exhibit reduced temporal flexibility and clockwatching. Based on available neuroimaging evidence in the healthy brain (2, 4, 5, 11, 13, 15–17, 20, 25, 28, 59–61), we further hypothesized that alterations of temporal awareness would have neural network correlates, reflecting the relative degree of involvement of posterior temporo-parietal cortices and hippocampus (engaged in temporal sequencing) vs. prefrontal and antero-mesial

temporal cortices (engaged in temporal scheduling, appraisal, and valuation).

METHODS

Participants

Seventy-one patients with FTD (34 bvFTD, 17 SD, 20 PNFA), twenty-eight patients with a typical memory-led syndrome of AD (hereafter, AD) and nine patients with LPA were recruited via a specialist cognitive disorders clinic. Thirty-two age-matched healthy individuals with no history of neurological or active psychiatric illness were recruited via the departmental research database. All patients fulfilled consensus diagnostic criteria for the relevant syndromic diagnosis (62–64) and all had clinically mild to moderate severity disease. Genetic screening revealed pathogenic mutations in twenty-two cases (eight *C9orf72*, all bvFTD; seven *MAPT*, 6 bvFTD and 1 SD; seven *GRN*, 4 bvFTD and 3 PNFA). Brain MRI was consistent

TABLE 1 | General demographic, clinical and neuropsychological characteristics of participant groups.

Characteristics	Controls	bvFTD	SD	PNFA	LPA	AD
General demographic and clinical						
No. (M/F)	32 (16/16)	34 (26/8)	17 (10/7)	20 (10/10)	9 (8/1)	28 (13/15)
Age (y)	68.2 (6.9)	65.8 (6.9)	66.5 (7.5)	68.5 (8.4)	69.2 (9.6)	70.4 (7.8)
Handedness (R/L)	29/2	32/1	17/0	18/1	8/1	25/2
Education (y)	16.1 (2.4)	13.8 (4.0)	15.0 (2.9)	13.6 (2.5)	16.2 (2.1)	14.9 (2.0)
MMSE (/30)	29.8 (0.4)	22.4 (6.4)	21.8 (8.0)	18.4 (9.5)	13.1 (7.8)	18.1 (6.6)
Symptom dur (y)	N/A	7.2 (5.0)	6.1 (2.4)	4.3 (2.4)	5.2 (1.9)	6.9 (3.6)
Medication use**: no (%)	2 (0.6)	16 (47)	6 (35)	8 (40)	2 (22)	13 (46)
Neuropsychological						
General intellect						
WASI VIQ	123.7 (8.2)[a]	**82.4 (27.6)[c]**	**67.9 (18.3)[a]**	**72.3 (18.9)[b]**	**61.3 (19.1)**	**93.1 (20.2)[a]**
WASI PIQ	125.2 (12.9)[a]	**92.8 (22.9)[c]**	114.5 (17.5)[a]	**88.8 (22.4)[b]**	**81.7 (12.9)**	**82.6 (16.7)[a]**
Episodic memory						
RMT Words (/50)	48.8 (1.2)[a]	**28.4 (18.8)[g]**	**26.3 (16.3)[d]**	**31.1 (18.4)[b]**	**20.2 (20.3)**	**25.4 (11.5)[e]**
RMT Words (/25)*	24.7 (0.8)	N/A	N/A	N/A	N/A	**15.3 (3.5)**
RMT Faces (/50)	43.9 (5.0)[a]	**23.7 (15.8)[f]**	**26.9 (11.7)[b]**	**30.5 (15.8)[b]**	**19.9 (19.7)**	**26.7 (11.7)[e]**
RMT Faces (/25)*	24.6 (0.7)	N/A	N/A	N/A	N/A	**17.8 (2.8)**
Executive function						
DS-F (max)	7.2 (1.1)[a]	**5.7 (1.4)[a]**	6.6 (0.9)[a]	**3.8 (2.2)[c]**	**3.0 (2.4)**	**5.9 (1.4)[a]**
DS-R (max)	5.5 (1.3)[a]	**3.6 (1.8)[a]**	5.0 (1.5)[a]	**1.9 (1.6)[c]**	**1.8 (1.4)**	**3.3 (1.8)[b]**
D-KEFS Stroop:						
Color (s)	30.1 (4.9)[b]	**53.8 (22.0)[a]**	**52.6 (23.2)[a]**	**77.3 (19.8)[d]**	**81.9 (13.7)**	**57.4 (17.1)[d]**
Word (s)	23.3 (5.0)[b]	**35.5 (19.0)[a]**	32.0 (18.4)[a]	**70.3 (25.5)[d]**	**60.8 (22.8)**	**44.4 (22.5)[d]**
Interference (s)	54.8 (13.2)[b]	**119.9 (54.8)[a]**	**96.9 (45.9)[a]**	**155.9 (44.8)[d]**	**180.0 (0.0)**	**145.6 (40.5)[d]**
Fluency:						
Verbal (total)	17.7 (5.7)[a]	**6.6 (5.4)[a]**	**6.9 (5.4)[a]**	**4.1 (4.4)[d]**	**2.0 (2.9)**	**8.9 (4.9)[b]**
Category (total)	24.6 (5.4)[a]	**10.0 (6.9)[a]**	**5.3 (4.4)[a]**	**9.4 (7.2)[d]**	**2.2 (2.9)**	**8.0 (5.0)[a]**
TMT A (s)	31.1 (9.2)[a]	**75.7 (46.4)[a]**	53.5 (27.7)[a]	**82.1 (45.4)[d]**	**116.9 (37.6)**	**99.3 (42.4)[a]**
TMT B (s)	60.2 (24.1)[a]	**202.2 (93.5)[a]**	**147.3 (88.4)[a]**	**229.2 (94.4)[c]**	**300.0 (0.0)**	**269.7 (69.0)[c]**
Language skills						
BPVS (/150)	148.0 (1.4)[a]	**110.7 (45.5)[e]**	**62.1 (39.8)[b]**	**117.6 (44.4)[b]**	**92.6 (55.0)**	**124.1 (36.7)[a]**
GNT (/30)	26.6 (2.7)[a]	**12.1 (9.3)[d]**	**1.0 (4.0)[a]**	**10.7 (7.2)[c]**	**7.0 (8.5)[a]**	**12.5 (8.3)[b]**
Other skills						
VOSP (/20)	18.9 (1.2)[a]	**14.2 (5.2)[d]**	**14.1 (4.7)[a]**	**15.1 (4.7)[b]**	**13.6 (3.7)**	**15.4 (2.6)[a]**

*Mean (standard deviation) values are shown unless otherwise indicated (maximum scores on neuropsychological tests are in parentheses); significant differences in performance between healthy controls and patient groups ($p < 0.05$) are coded in bold. *based on data from an historical cohort of 24 healthy older controls and six patients with AD from the present cohort; **includes medications with a potentially relevant effect on time perception (see text). A reduced number of participants completed certain tests, as follows: [a]n-1, [b]n-2, [c]n-3, [d]n-4, [e]n-7, [f]n-9, [g]n-11. AD, patient group with typical Alzheimer's disease; BPVS, British Picture Vocabulary Scale (67); bvFTD, patient group with behavioral variant frontotemporal dementia; Controls, healthy control group; D-KEFS, Delis Kaplan Executive System (68); dur, duration; F, female; GDA, Graded Difficulty Arithmetic test (69); GNT, Graded Naming Test (70); LPA, patient group with logopenic aphasia; M, male; MMSE, Mini-Mental State Examination score (71); N/A, not applicable; PIQ, performance IQ; PNFA, patient group with progressive non-fluent aphasia; RMT, Recognition Memory Test (72); SD, patient group with semantic dementia; TMT, Trails Making Test (73); VIQ, verbal IQ; VOSP, Visual Object and Space Perception Battery – Object Decision test (74); WASI, Wechsler Abbreviated Scale of Intelligence (75); DS-F/R, Wechsler Memory Scale (Revised) Digit Span – Forward/Reverse (76); y, years.*

with the syndromic diagnosis in all patients and none had evidence of significant cerebrovascular burden. Cerebrospinal fluid profiles of tau and beta-amyloid$_{1-42}$ were available for twelve patients with typical AD and six patients with LPA; each was consistent with underlying Alzheimer's pathology, based on local reference ranges (total tau/beta-amyloid$_{1-42}$ ratio >0.8). In all patients, the syndromic diagnosis was further corroborated by a comprehensive general neuropsychological assessment. We recorded the use of medications (antidepressants and neuroleptics) that could potentially affect time perception among the participant groups (65, 66). Clinical, demographic, and neuropsychological characteristics of all participant groups are summarized in **Table 1**.

All participants gave informed consent for their involvement in the study. Ethical approval was granted by the University College London and National Hospital for Neurology and Neurosurgery Joint Research Ethics Committees in accordance with Declaration of Helsinki.

Assessment of Temporal Awareness

We surveyed the presence of behavioral symptoms suggesting an alteration of subjective temporal awareness (**Table 2**). We sampled temporal behavioral symptoms that we felt were likely to be pertinent based on our accumulated clinical experience of the target syndromes. These symptoms comprised: apparent confusion about the temporal ordering of experienced past personal events and/or how long ago such events occurred or will occur in future (i.e., difficulty estimating the interval separating the present from the past/prospective event); reduced temporal flexibility (temporal rigidity, exemplified by high valuation of punctuality, and discomfort if schedules were disturbed) and/or clockwatching (looking at their watch or asking for the time very often); and an increased tendency to re-live personal events from the past (as indicated, for example, by a conversational preoccupation with such events). The survey was completed by healthy controls and by each patient's primary caregiver; involvement of caregivers (either the patient's spouse or child) was intended to maximize understanding, communication, and accuracy of symptom reporting, since people with dementia (particularly FTD syndromes) often have limited insight into their own illness. For each of the sampled symptoms of altered temporal awareness, survey respondents were asked to indicate whether or not prominent changes (i.e., evident most days) had occurred. Caregivers were asked to compare patients' current behavior with their behavior premorbidly while healthy controls were asked whether they felt there had been any changes in their own behavior, referenced in each case to the situation 10 years previously: this interval reflects the typical duration of clinical symptoms in the target diseases plus some allowance for any prodromal changes. In addition, respondents were given the opportunity to make free comments, to provide further details about temporal behavioral alterations.

TABLE 2 | Survey used to identify alterations in temporal awareness.

Temporal symptom	Questions
	Thinking about [her / his / your] activities most days, please indicate whether or not you feel there has been a clear increase in any of the following
Ordering past events	Confusion about the order in which personal events have happened
Estimating intervals between events	Difficulty estimating how long ago personal events occurred/how far in the future events will occur
Temporal rigidity	Intolerant of delays, anxiety or irritation about missing appointments or late arrivals, insistence on doing things at a particular time
Clockwatching	Tendency to "watch the clock" or preoccupation with the time
Re-living past events	Tendency to re-live personal events or episodes from the past

Survey symptom items were chosen based on clinical observations of target disease groups and informed by previous studies of temporal awareness in the healthy brain (see text). The questionnaire was completed by healthy control participants themselves and by patients' primary caregivers. Respondents were asked to indicate whether or not prominent changes (i.e., evident most days) had occurred, for each of the sampled symptoms of temporal awareness. Caregivers were asked to decide whether changes had occurred comparing patients' current behavior with their behavior when well; healthy controls were asked to decide if changes had occurred in their own behavior over the past 10 years.

Analysis of Clinical and Behavioral Data

Clinical and behavioral data were analyzed using Stata version 14.0 software (StataCorp, College Station, TX, USA). Participant groups were compared using a one-way ANOVA for continuous variables satisfying normality criteria, or the non-parametric equivalent Kruskal-Wallis test if this was not the case. *Post-hoc* tests of non-parametric continuous variables were performed using Dunn's test. For categorical variables, we used the chi-square test, or Fisher's exact test when expected counts were small.

Survey data were analyzed to determine the prevalence of changes in temporal awareness for each participant group. Temporal awareness symptoms were coded as 1 (present) or 0 (absent). Because for three of the five symptom items no healthy control participant exhibited the symptom, we did not build a logistic regression model to compare the prevalence of alterations on that item for all patient groups vs. the healthy control group. Instead, we performed a two-tailed Fisher's exact test for each item and corrected for multiple comparisons using the Benjamini-Yekutieli procedure (77). For each temporal awareness symptom, once we had established an overall disease effect for that symptom, we used a logistic regression model to compare the log odds of exhibiting that symptom (as the dependent variable) between patient groups. We specified a dummy variable for diagnosis (our main variable of interest) taking the AD group as the reference and included age, gender and Mini-Mental State Examination (MMSE) score as covariates, to take into account of potentially confounding effects from these factors. MMSE score here served as an index of overall disease severity; although there is no single, principled index of severity for FTD syndromes (and all candidate severity measures are to some extent problematic and potentially confounded by linguistic and other considerations), the MMSE is a simple, widely used index that can be applied across participant groups.

We also built logistic regression models to assess possible correlations between temporal awareness symptoms. Finally, we looked for any associations between altered temporal awareness and general clinical covariates (age, gender, years of education, MMSE score, relevant medication use) using the Student's *t*-test or the Wilcoxon rank sum test for continuous variables, and the chi-square test for categorical variables. A statistical significance threshold $p < 0.05$ was accepted for all tests.

Brain Image Acquisition and Analysis

Volumetric brain MRI data from 91 patients were entered into the VBM analysis; scans were unavailable for 14 patients (three bvFTD, one PNFA, two LPA, eight AD) and a further three (two bvFTD, one PNFA) were inadequate on technical grounds. For each patient, a sagittal 3D magnetization-prepared rapid-gradient echo T1-weighted volumetric brain MR sequence (TE/TR/TI 2.9/2200/900 ms, dimensions 256 × 256 × 208, voxel volume of 1.1 × 1.1 × 1.1 mm) was acquired on a Siemens Prisma 3T MRI scanner using a 32-channel phased array head-coil. Pre-processing of brain images was performed using the New Segment and Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) toolboxes in SPM12 (www.fil.ion.ucl.ac.uk/spm/software/spm12/), following an optimized protocol (78). Normalization, segmentation, and modulation of gray and white matter images were carried out using default parameter settings. Gray matter images were subsequently smoothed using a 6 mm full width-at-half-maximum Gaussian kernel. For each patient, total intracranial volume was calculated by combining gray matter, white matter, cerebrospinal fluid volumes after segmentation of these tissue types. A study-specific template brain image was created by warping all native space whole-brain images to the final DARTEL template and calculating the average of the warped brain images.

Across the combined patient cohort, we ran a full factorial model to assess associations of regional gray matter volume with each temporal symptom. The model incorporated the five temporal symptom items (coded as 0/1 for absence/presence of that symptom, respectively), diagnosis as a five-level factor, as well as age, total intracranial volume, and MMSE score as nuisance covariates. Negative (inverse) associations with regional gray matter (i.e., associations with gray matter atrophy) were assessed for every symptom item; positive gray matter associations were additionally assessed for symptoms of temporal rigidity, clockwatching, and re-living the past, since these phenomena are likely *a priori* to require at least partially preserved temporal processing mechanisms (58). Statistical parametric maps were generated using an initial threshold $p < 0.001$ and evaluated at peak voxel statistical significance level $p < 0.05$, after family-wise error (FWE) correction for multiple voxel-wise comparisons, separately within individual pre-specified neuroanatomical regions of interest. These regions were selected *a priori* based on functional neuroanatomical substrates of subjective time awareness identified in the healthy brain comprising anterior temporal lobe (the anterior parts of the superior, middle, inferior temporal, and fusiform gyri, and the temporal pole) (27, 79–81), insular cortex (2, 5, 13, 82), parietal cortex (inferior and superior parietal lobules, precuneus, and posterior cingulate cortex) (14–17, 83) and hippocampus (3, 19, 20). Regions were defined for the right and left hemispheres using the Harvard-Oxford Brain Atlas (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases). The regions used are presented in **Figure S1**.

RESULTS

General Clinical and Neuropsychological Data

Participant groups (see **Table 1**) did not differ in age [$F(5,134) = 1.31$; $p = 0.2619$], nor handedness ($p = 0.885$); but differed significantly in gender balance [$X^2(5,140) = 11.204$; $p = 0.047$] and years of education [$X^2(5,138) = 15.212$; $p = 0.0095$]; the absolute difference was small, of the order of two years). Syndromic groups did not differ significantly in clinical illness duration [$X^2(4,103) = 8.43$; $p = 0.077$]. However, there was a significant difference in MMSE scores [$F(4,103) = 3.66$; $p = 0.0078$], attributable in a *post hoc* analysis to significantly higher scores in the bvFTD group compared with the LPA group ($p = 0.012$).

Temporal Awareness Symptom Data

Data on the prevalence of temporal awareness alterations for all participant groups are summarized in **Table 3**. Examples of caregiver comments about patients' behavioral alterations are provided in **Table S1**. The logistic regression analysis comparing syndromic groups is presented in **Table 4**. Logistic regression analysis probing temporal symptom correlations are also presented in **Table S2**. Prevalence data for altered temporal awareness in individual patients with genetic mutations causing FTD are summarized in **Table S3**.

Raw prevalence data (**Table 3**) indicated that alterations of temporal awareness were frequent in all syndromic groups but experienced by only a small minority of healthy controls. Overall, patients with probable AD pathology (AD and LPA) most frequently exhibited confusion ordering past events or difficulty estimating intervals between events; while patients with FTD pathology (bvFTD, SD, PNFA) were most frequently prone

TABLE 3 | Proportions of participant groups with altered time awareness.

Temporal symptom	Controls	bvFTD	SD	PNFA	LPA	AD
	n = 32	*n* = 34	*n* = 17	*n* = 20	*n* = 9	*n* = 28
Ordering past events	0%	62%	12%	15%	56%	68%
Estimating intervals between events	0%	59%	18%	35%	67%	79%
Temporal rigidity	0%	41%	65%	35%	11%	11%
Clockwatching	3%	44%	59%	35%	22%	18%
Re-living past events	9%	59%	47%	25%	22%	21%

*Data derived from the customized questionnaire (see text and **Table 2**) were used to calculate the prevalence of time awareness alterations in each participant group, summarized here as proportion (percentage) of the group showing an alteration (total group sizes shown above). All patient groups combined were significantly different from the healthy control group ($p < 0.001$ after correction for multiple comparison).*

TABLE 4 | Results of the logistic regression analysis over the patient cohort.

Temporal symptom	Variable	OR	95% CI	*P*-value
Ordering past events	Diagnosis			
	bvFTD	0.94	0.28–3.12	0.926
	SD	0.06	0.01–0.35	**0.002**
	PNFA	0.06	0.01–0.30	**0.001**
	LPA	0.34	0.06–1.91	0.219
	Gender (F)	0.91	0.33–2.50	0.852
	Age	0.97	0.92–1.04	0.407
	MMSE	0.92	0.86–0.98	**0.010**
	Constant	71.55	0.77–6633.90	0.065
Estimating intervals between events	Diagnosis			
	bvFTD	0.51	0.14–1.82	0.302
	SD	0.06	0.01–0.31	**0.001**
	PNFA	0.12	0.03–0.49	**0.003**
	LPA	0.33	0.05–2.02	0.232
	Gender (F)	1.03	0.39–2.71	0.948
	Age	0.99	0.93–1.05	0.666
	MMSE	0.91	0.86–0.97	**0.005**
	Constant	51.48	0.62–4292.08	0.081
Temporal rigidity	Diagnosis			
	bvFTD	5.50	1.24–24.40	**0.025**
	SD	17.33	3.30–91.09	**0.001**
	PNFA	5.29	1.11–25.14	**0.036**
	LPA	1.04	0.09–12.41	0.975
	Gender (F)	0.52	0.19–1.41	0.197
	Age	1.05	0.99–1.11	0.132
	MMSE	1.04	0.98–1.11	0.203
	Constant	0.00	0.00–0.29	**0.013**
Clockwatching	Diagnosis			
	bvFTD	4.44	1.21–16.35	**0.025**
	SD	8.98	2.09–38.58	**0.003**
	PNFA	2.80	0.70–11.12	0.146
	LPA	0.90	0.13–6.41	0.919
	Gender (F)	0.48	0.18–1.24	0.130
	Age	1.06	1.00–1.12	0.054
	MMSE	10.98	0.92–1.04	0.450
	Constant	0.01	0.00–0.57	**0.026**
Re-living past events	Diagnosis			
	bvFTD	3.49	1.05–11.63	**0.042**
	SD	2.40	0.61–9.47	0.212
	PNFA	1.12	0.28–4.47	0.875
	LPA	1.06	0.16–6.93	0.951
	Gender (F)	0.66	0.26–1.66	0.378
	Age	0.97	0.91–1.02	0.256
	MMSE	1.04	0.98–1.11	0.154
	Constant	1.56	0.03–96.61	0.832

The Alzheimer's disease group is the reference for comparisons between diagnostic groups; significant associations with particular variables ($p < 0.05$) are coded in bold. bvFTD, patient group with behavioral variant frontotemporal dementia; CI, confidence interval; F, female; LPA, patient group with logopenic progressive aphasia; MMSE, Mini-Mental State Examination score; OR, odds ratio; PNFA, patient group with progressive non-fluent aphasia; SD, patient group with semantic dementia.

to temporal rigidity, clockwatching and/or a tendency to re-live past events. Certain temporal symptoms were especially salient in particular syndromic groups (present in over half the cases in that group): event ordering confusion and/or difficulty estimating intervals between events in bvFTD, LPA, and AD; temporal rigidity and clockwatching in SD; and a tendency to re-live past events in bvFTD. Difficulties with interval estimation was significantly correlated with temporal rigidity, whereas no significant associations were found between these symptoms and a tendency to re-live past events (**Table S2**).

Compared to healthy controls, the patient cohort overall had a significantly higher prevalence of confusion about ordering events in time and difficulty estimating intervals between events (both $p < 0.0001$). In the logistic regression analysis comparing syndromic groups, there was a main effect of diagnosis for both confusion of temporal order [$X^2(4, 103) = 20.70$, $p = 0.0004$] and interval estimation difficulties [$X^2(4, 103) = 16.29$, $p = 0.0026$]. Such disturbances were significantly more prevalent in the AD group than the SD group [temporal ordering: odds ratio (OR) = 0.06, 95% confidence interval (CI) 0.01–0.35; temporal estimation: OR = 0.06, CI 0.01–0.31] and the PNFA group (temporal ordering: OR = 0.06, CI 0.01–0.30; temporal estimation: OR = 0.12, CI 0.03–0.49).

Compared to healthy controls, the patient cohort overall also had a significantly higher prevalence of increased temporal rigidity and clockwatching (both $p < 0.001$). There was a main effect of diagnosis for clockwatching [$X^2(4, 103) = 10.57$, $p = 0.0318$] and temporal rigidity [$X^2(4, 103) = 12.98$, $p = 0.0114$]. Clockwatching was significantly more prevalent in the bvFTD and SD groups than the AD group (bvFTD: OR = 4.44, CI 1.21–16.35; SD: OR = 8.98, CI 2.09–38.58). Temporal rigidity was significant more prevalent in the bvFTD, SD, and PNFA groups than the AD group (bvFTD: OR = 5.50, CI 1.24–24.40; SD: OR = 17.33, CI 3.30–91.09; PNFA: OR = 5.29, CI 1.11–25.14).

Compared to healthy controls, patient groups overall were significantly more likely to re-live past events ($p < 0.001$). However, while this symptom was more prevalent in the bvFTD and SD groups, the logistic regression analysis showed no significant main effect of diagnosis [$X^2(4,108) = 5.78$, $p = 0.22$], precluding further comparisons between disease groups.

Across the patient cohort, symptoms of disturbed past event ordering, or interval estimation were significantly associated with MMSE score (OR = 0.92, CI 0.86–0.98 and OR = 0.91, 95% CI 0.86–0.97, respectively). No other significant associations between developing temporal awareness symptoms and general patient characteristics (gender, age, education, or relevant medication use) were identified.

Considering the small subgroup of patients with genetic mutations (**Table S3**), symptoms of altered time awareness were generally frequent with all major mutations causing FTD. However, temporal rigidity was particularly associated with *MAPT* mutations, contrasting with its low prevalence in association with *C9orf72* and *GRN* mutations; no patients with *GRN* mutations were reported to have exhibited disturbances of temporal event ordering or interval estimation.

TABLE 5 | Neuroanatomical associations of altered time awareness in the patient cohort.

Region	Side	Cluster (voxels)	Peak (mm)			T score	P_{FWE}
			x	y	z		
Middle temporal gyrus/superior temporal sulcus	L	118	−50	−3	−26	3.95	0.038
Hippocampus	L	31	−22	−33	−4	3.74	0.019
Posterior cingulate	L	184	−2	−24	33	4.52	0.015
Superior parietal lobule	L	75	−32	−57	51	4.23	0.038

*The table presents the locations of regional gray matter positively correlated with tendency to re-live past events (the only significant association of altered time awareness identified) over the combined patient cohort, based on voxel-based morphometry (see text and **Tables 2**, **3** for further details). Coordinates of local maxima are in standard MNI space. P-values were all significant ($p < 0.05$) after family-wise error (FWE) correction for multiple voxel-wise comparisons within pre-specified anatomical regions of interest (see text).*

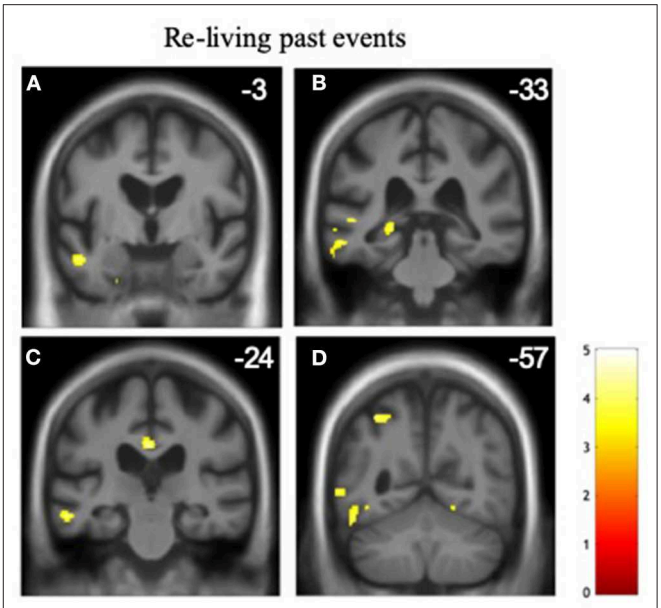

FIGURE 1 | Gray matter associations of altered temporal awareness in the patient cohort. Statistical parametric maps show regional gray matter volume positively associated with the propensity to re-live past events across all syndromic groups (see text and **Tables 2**, **3** for details). For display purposes, maps have been rendered on coronal sections of the group mean template T1-weighted MR brain image and thresholded at $p < 0.001$, uncorrected for multiple voxel-wise comparisons over the whole brain volume (areas significant at $p_{FWE} < 0.05$ after correction for multiple comparisons within pre-specified neuroanatomical regions of interest are summarized in **Table 5**); the y-coordinate (mm) in MNI space of the plane of each section is indicated. The color bar codes voxel-wise T scores. The left hemisphere is shown on the left in all sections. The sections traverse the following key structures: **(A)** anterior middle temporal gyrus/superior temporal sulcus. **(B)** posterior hippocampus. **(C)** posterior cingulate cortex. **(D)** superior parietal lobule.

Neuroanatomical Associations of Altered Time Awareness

Significant gray matter associations of altered time awareness across the patient cohort are summarized in **Table 5**, all thresholded at $p_{FWE} < 0.05$ within pre-specified anatomical regions of interest; statistical parametric maps are presented in **Figure 1**. Across the combined patient cohort, increased tendency to re-live past events was associated with relatively preserved gray matter in a distributed left-sided network including anterior middle temporal gyrus and superior temporal sulcus, hippocampus, posterior cingulate, and superior parietal cortices. No other neuroanatomical associations of altered temporal awareness were identified.

DISCUSSION

Here we have demonstrated alterations of multiple dimensions of long-duration, subjective time awareness in canonical syndromes of FTD, referenced to syndromes of AD as well as healthy older individuals. Abnormalities of time awareness were exhibited by over half of patients with bvFTD and SD as well as typical amnestic AD and LPA, and by around a third of patients with PNFA. However, the pattern of abnormalities was not uniform over the patient cohort: syndromic groups showed separable (albeit overlapping) profiles of altered time awareness, in line with our prior clinical hypotheses. Patients with typical AD and LPA had particularly salient difficulties with ordering past events and placing events in time, without significant changes in other aspects of temporal behavior sampled here; the pattern of temporal symptoms was much more heterogeneous in the FTD cohort. The SD group had prominent temporal rigidity and clockwatching, while the bvFTD group exhibited predominant abnormalities of past event ordering and re-living past events. Profiles of altered temporal awareness differed significantly between AD and FTD disease groups when directly compared and although there was an overall correlation of abnormal past event ordering and interval estimation with worsening disease severity, syndromic profiles of altered temporal awareness were evident after taking background clinical characteristics (age, gender, MMSE score) into account.

Informed by previous work in the healthy brain (84–87), we propose a tentative synthesis of these findings in terms of different "domains" of temporal awareness. Symptoms of abnormal event ordering or interval estimation might both plausibly reflect a disturbed mental timeline, while temporal rigidity and clockwatching reflect related aspects of mental timekeeping. The latter might be further related to the "Godot syndrome", a phenomenon previously described in AD, and which refers to anxiety surrounding upcoming events (88, 89). Our findings corroborate previous reports of obsessional clockwatching in SD and bvFTD (31, 58) as well as time estimation difficulties in AD (46) but further illustrate that alterations of temporal awareness transcend canonical syndromic boundaries. Across syndromic groups, patients presenting with temporal rigidity, or clockwatching were less likely to also exhibit difficulty ordering past events and vice versa, but no association was found between those symptoms and a tendency to re-live past events. This is in line with the hypothesis that the propensity to re-live past events might constitute a partly compensatory phenomenon in the face of impoverished mental

timeline, somewhat analogous to the normal phenomenon of "nostalgia" (77, 90).

Across the combined patient cohort, a tendency to re-live past events was associated with relative preservation of a distributed left-sided temporal, parietal, and hippocampal network. This is in line with an emerging picture of temporal processing and subjective temporal awareness derived from studies of the healthy brain. In particular, the parietal cortex has been implicated in reconstructing sequences of spatio-temporal events and their temporal ordering (15, 16, 91, 92). In conjunction with hippocampus as well as insular and prefrontal cortices, the parietal lobes participate in a distributed neural network that accesses and manipulates the mental timeline according to salience and behavioral context (82, 93, 94). This network further overlaps core elements of the default mode network which integrates information about current bodily states and memories with incoming sensory traffic. Moreover, the hippocampus is critical for initial encoding of events and their embedding in emotional context (3, 19, 20, 95), and the anterior temporal lobe is likely to be important for the semantic integration of autobiographical events (96). The integrity (or partial integrity) of these brain areas might plausibly support a tendency to re-live past autobiographical events, in line with previous evidence in neurodegenerative syndromes (97–99).

The lack of gray matter associations of other symptoms of altered temporal awareness here is, at first sight, a little surprising. However, there are several factors that might potentially account for these results. Firstly, the neurobiological status of the temporal awareness "symptoms" we considered here are different between symptom categories. Re-living of past events depends to some extent on a neuroanatomical substrate that is at least partly preserved (since temporal events have to be represented and accessed in order to be re-lived). It is therefore plausible that this substrate should be identified across syndromic groups, since it reflects the architecture of the healthy brain. On the other hand, we sampled other temporal symptoms that directly reflect the impact of neurodegenerative brain damage, which will have varied between syndromes and is therefore less likely to have a common neuroanatomical association across the patient cohort. In addition, it is possible that the neuroanatomical substrates of these other temporal symptoms are extensively distributed, widely variable between individuals or alternatively, highly convergent between symptom categories: any of these scenarios would have made neuroanatomical associations less liable to be identified using the VBM model we employed here. It is further plausible that at least some symptoms may arise from network connectivity changes that are not captured using VBM. Finally, the binarised symptom classification here may well have reduced scope to detect gray matter associations that might have been evident with a continuously distributed variable (e.g., a symptom severity score). It should be kept in mind that these factors are, to some extent, limitations imposed by the VBM technique itself (which is essentially a correlational methodology) rather than specific to temporal processing per se.

Furthermore, cellular and molecular as well as macro-anatomical factors are likely to influence temporal processing and subjective temporal awareness in neurodegenerative pathologies (100). The small case numbers here preclude firm conclusions concerning the temporal awareness profiles of particular genetic mutations. However, it is noteworthy that temporal rigidity and clockwatching were not reported in patients with *GRN* mutations, whereas these were salient symptoms in patients with *MAPT* mutations. It may be relevant that *MAPT* mutations target the antero-mesial temporal lobes relatively selectively (101) while *GRN* mutations frequently target parietal cortex (102). These issues will only be resolved by further neuroanatomical work addressing functional as well as structural network connectivity.

From a clinical perspective, our findings endorse the long-held bedside impression of temporal obsessionality in SD and bvFTD, while further corroborating reports of disordered temporal estimation in AD. Quantification of changes within the temporal symptom categories we have foregrounded here would help in planning, implementing and evaluating new behavioral interventions designed to help patients orient and navigate in time and to reduce patient and caregiver distress incurred by abnormal temporal reactivity. The overall correlation of mental timeline abnormalities with advancing disease noted here accords with previous suggestions that clockwatching behavior may be restricted to the earlier stages of SD (58). Our observations here could motivate further work to develop quantifiable cognitive tests of temporal awareness. Such tests might, in future studies, yield novel functional biomarkers that index the integrity of temporal processing mechanisms in neurodegenerative syndromes.

This study has several limitations that should inform future work. Most fundamentally, there is a need to further corroborate the results in larger patient cohorts and ideally with histopathological and molecular correlation. It would be of considerable interest to compare the profiles of altered temporal awareness exhibited by patients with FTD and AD directly with other neurodegenerative pathologies, for example Lewy body disease, which also target brain systems implicated in temporal perception. It will also be relevant to study these profiles longitudinally: the phenomenology of neurodegenerative syndromes is dynamic and multiphasic, while both in patients and healthy individuals, subjective temporal awareness may be modulated by key life events (such as retirement from work). The categories of temporal symptoms assessed in this first study were intentionally broad and qualitative, designed to capture a diverse range of phenomena. However, these symptom categories should be unpacked in further studies to quantify the frequency and severity of symptoms that patients experience and to capture the nature of their difficulties more precisely. For example, "difficulty" estimating the temporal intervals between events could mean simply that patients fail to take account of lapsed time or rather that they express unreasonable estimates of the relevant intervals. In turn, these processes might plausibly be affected differentially in different syndromes and diseases (in particular, AD vs. FTD). Particularly with regard to symptoms suggesting disturbances of the mental timeline, it will be crucial to define how such disturbances relate to deficits of episodic memory and the detail with which particular events and their spatio-temporal context are encoded. It will also be important to acquire patients' own reports of time awareness,

ideally in parallel with caregivers' perspectives. Certain aspects of temporal awareness (for example, capacity to envisage the future) are intrinsically difficult to capture from second-person questionnaires but potentially highly illuminating in particular neurodegenerative syndromes [such as bvFTD and SD (53, 55)]. Our findings provide a prima *facie case* for the future design and validation of temporal symptom scales relevant to a broad range of neurodegenerative diseases.

Understanding the neural mechanisms of altered temporal awareness associated with these neurodegenerative pathologies will require a detailed assessment of temporal perception (in particular the psychophysical correlates of interval and pattern processing), consideration of accompanying behavioral phenotypes [since altered emotional reactivity is very likely to impact on temporal behaviors (7)], and functional neuroimaging techniques that can capture dynamic interactions and connectivity between brain network elements. In this regard, magnetoencephalography would be a particularly attractive modality, by virtue of its high temporal resolution and capacity to track changes in cortical laminar physiology. The last would offer the exciting prospect of relating complex temporal behavioral phenotypes to dysfunction at the level of tissue microcircuits and synapses, which is likely to be apposite in light of emerging evidence that neural mechanisms of temporal awareness span scales ranging from the cellular to the macroscopic (4, 5). Indeed, this is arguably a compelling motivation for developing true "temporal biomarkers," since indices of universal brain processes (such as temporal processing) are likely to prevail across pathologies and disease stages.

This preliminary study calls attention to the significance of temporal awareness symptoms and pertinent neural network substrates across major dementia syndromes. The findings provide a rationale for a more systematic analysis of subjective time in neurodegenerative pathologies, with a view to developing validated clinical assessment tools, understanding underlying neurobiological mechanisms and designing management interventions.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University College London and National Hospital for Neurology and Neurosurgery Joint Research Ethics Committees. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CM and JW were responsible for the conception and design of the study. CM and HS carried out symptom survey data collection. LR, CG, KM, EB, and JR were responsible for recruitment, neuropsychology, and imaging data acquisition. M-CR-K was responsible for the analysis of the survey data and M-CR-K, CM, CH, RB, and JA for the voxel-based morphometry analysis. M-CR-K, CM, and JW were responsible for the interpretation of the data. M-CR-K and JW were responsible for drafting the manuscript. M-CR-K, CM, CH, and JW for revisiting it critically for important intellectual content. All authors read and approved the final manuscript.

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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.2020.00291/full#supplementary-material

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Validation of the RUDAS for the Identification of Dementia in Illiterate and Low-Educated Older Adults in Lima, Peru

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Objectives: To evaluate the performance of the Peruvian version of the Rowland Universal Dementia Assessment Scale (RUDAS-PE) in discriminating between controls and patients with mild cognitive impairment (MCI) and dementia in an illiterate population with low-levels of education.

Methods: We compared the cognitive performance of 187 elderly subjects who were illiterate (controls $n = 60$; MCI $n = 64$; dementia $n = 63$). Neuropsychological measures included the RUDAS-PE, Mini-Mental State Examination (MMSE), INECO Frontal Screening (IFS), and Pfeffer Functional Activities Questionnaire (PFAQ). The results were compared to a neuropsychological evaluation (gold standard), including use of Clinical Dementia Rating (CDR) scores.

Results: We found a Cronbach's alpha was 0.65; Spearman's correlation coefficient was 0.79 ($p < 0.01$). The area under the receiver operating characteristics curve for the RUDAS to discriminate dementia from MCI was 98.0% with an optimal cut-off <19 (sensitivity 95%, specificity 97%); whereas, to differentiate MCI and controls was 98.0% with an optimal cut-off <23 (sensitivity 89%, specificity 93%).

Conclusions: Based on its excellent psychometric properties, we find the RUDAS-PE suitable to aid in the opportune detection of dementia in a geriatric illiterate population with low-levels of education.

Keywords: mild cognitive impairment, neuropsychology, dementia, neurocognitive disorders, Alzheimer's disease, brief cognitive assessment, illiteracy

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INTRODUCTION

Illiteracy rates among youth (age 15 to 24 years) and adults are decreasing worldwide. In Peru, data from the Instituto Nacional de Estadística e Informática (INEI) show that illiteracy rates among persons age 15 years and older remain high: 5.9% (1). Moreover, illiteracy among adults age 60 years and older are highest in rural areas (41.6% rural vs. 12.3% urban) and higher among females

(27.5 vs. 8.2% in males) (2). Studies show that 21.9% of the population in Peru has an elementary level of education; the majority of which is found among rural inhabitants (43 vs. 16% urban) (2).

It is common to find large portions of the older population experience limited access to health care systems, whether as a result of age discrimination or other barriers such as cost. This raises a high concern as the health and medical needs for this age group, particularly in the ability to detect cognitive deterioration and dementia (3, 4), are largely unmet (5). In Peru, this is primarily due to a lack of validated and standardized instruments to evaluate cognition and functionality in marginalized populations, i.e., low-levels of education and literacy rates, rural communities, indigenous groups or populations where multiple languages exist in addition to Spanish (6, 7).

Many attempts have been made (4) with the purpose of detecting dementia in illiterate populations in low-educational settings. The Cognitive State Test (COST) seems to give acceptable results (3). Unfortunately, like the Mini-Mental State Examination (MMSE) (8–10), the COST fails to address mild cognitive impairment (4). In Peru, the Memory Alteration Test ($M^{@}T$) results have only been reported in individuals of low-educational backgrounds (at least 4 years of regular education) (11) and can distinguish patients with amnestic mild cognitive impairment (aMCI) from controls and patients with early stage Alzheimer's disease [AD]. Yet, since it can only evaluate memory and orientation, the $M^{@}T$ is unable to detect other types of dementia.

Within the framework of this criteria, we look to validate the Rowland Universal Dementia Assessment Scale (RUDAS). The RUDAS is a BCT that has proven itself a useful instrument for the detection of dementia in an illiterate population within a primary care setting (12) as well as in populations with a low-level of education (13, 14). It is a simple instrument, consisting of six-items that explore recent verbal memory, visuo-spatial orientation, motor praxis, visuo-constructive praxis, judgment, and language. Like the MMSE, it has an optimal score of 30 points, where lower scores suggest severe cognitive impairment (12). It has been proposed that the RUDAS has reasonable psychometric characteristics and is particularly useful in patients of various languages and cultures, thereby being preferable in populations with low-levels of educational attainment. While it is true that the RUDAS is validated in an urban Peruvian population with a middle-level of education (15), it has yet to be adapted for and evaluated in an illiterate elderly population with low-levels of education.

METHODS

Study Design

This is a diagnostic accuracy study designed focusing on an urban illiterate population with the following objectives:

1. Establish the sensitivity and specificity of the RUDAS.
2. Establish the parameters for the RUDAS to discriminate MCI and dementia.
3. Compare the capacity of the RUDAS and MMSE to discriminate between normal cognitive function and patients with MCI and dementia.

Participants

This study took place in regional health clinics within Lima, Peru. A previous awareness campaign on risk factors involved in cognitive impairment served as our primary source of patient recruitment within the Ventanilla community. Potential research participants included those who regularly assisted the scheduled activities designed to evaluate cognitive impairment as part of the pre-screening process. After having passed a screening test and neuropsychological evaluation, individuals were then allocated into three groups for further statistical analysis:

1. Control group: Individuals without cognitive impairment (no-CI) (CDR = 0).
2. Mild Cognitive Impairment (MCI) group: Individuals with clinical and neuropsychological criteria of MCI (CDR = 0.5).
3. Dementia group: Individuals with clinical and neuropsychological criteria compatible with dementia in its initial stages (CDR = 1 and 2).

Inclusion Criteria

1. Participants were selected according to the following criteria:
2. Males and females aged 60 and above.
3. Illiterate persons of at least 15 years old defined as one with no education (< 1 full year of formal education completed, and inability to read or write). Also, individuals without prior literacy experience who participate in basic adult education programs or "night school" on a regular basis after having turned 60).
4. Individuals who are native Spanish speakers or persons who speak Spanish as a second language > 10 years.
5. Individuals diagnosed with aMCI based on the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) and Clinical Dementia Rating (CDR) criteria.
6. Individuals diagnosed with signs of dementia according to DSM-5 and CDR criteria.

Exclusion Criteria

Participants were excluded if they had difficulty performing the cognitive tests due to auditory or visual problems, or any other physical problems that interfered with their performance; if they were considered functionally literate (defined as those who received a non-formal education for a minimum of 4 years before the age of 15, are able to read, write, do mathematical calculations, and are socially functional); or did not speak or understand Spanish. We further excluded patients who were diagnosed/or had symptoms compatible with advanced stage dementia or another psychiatric illness (bipolar disorder, psychosis, schizophrenia, and personality disorders) as well as participants diagnosed with concomitant cerebrovascular pathologies, mental retardation, traumatic brain injury sequelae, depression (according to the Beck Depression Inventory-II), had a history of addiction or substance abuse, or who in the last seven consecutive days prior to the evaluation had taken

any of the following medications: opioids, decongestants, anti-spasmodics, anti-cholinergics, anti-arrhythmic, anti-depressants, anti-psychotics, such as valproate, phenobarbital, fentanyl, carbamazepine, and levetiracetam. In cases where patients would take these medications for a chronic illness, and only if their medical condition would allow it, it was recommended to stop their medication for seven consecutive days prior to commencing the brief cognitive assessment.

Ethical Aspects

This study was conducted in accordance with the Council for International Organizations and Medical Sciences guidelines. All participants signed a consent form prior to the study in accordance with the Declaration of Helsinki. The protocol was approved by the ethics committee at the Instituto de Medicina Tropical "Daniel Alcides Carrión" of the "Universidad Nacional Mayor de San Marcos" approval number CIEI-2018-020.

Measures

Rowland Universal Dementia Assessment Scale (RUDAS)

Validation of the RUDAS in an urban population with a mid level education

The RUDAS has recently been evaluated in patients age ≥60 years with a mid-level education in Peru. The optimal cut-off for ED and MCI was <21 with a sensitivity of 90.2% and specificity of 73.8%; and <24 between MCI and controls with a sensitivity of 96% and specificity of 90.2% (15).

RUDAS-PE adaptation for illiterate population

The adaptation process corresponded to the standards and methodology of the RUDAS-PE set by Custodio et al. (15) and based on the Spanish translated version of the RUDAS by Ramos-Ríos et al. (14). Suggestions made by clinical experts (neurologists SC and DL, and neuropsychologists JC and MS) were used to make improvements to the existing RUDAS-PE. Only minor procedural changes to the administration of the test were made: (1) state the precise time allotted for the administrator to demonstrate and evaluate the alternating hands portion of the motor praxis section, and (2) instruct the test administrator on the specific size of the cube in the cube-drawing portion of the visuo-constructional section of the test as follows, "take a sheet of A4 paper and draw a cube with lateral edges of 12 cm in length at a 45° angle." These recommendations were approved and introduced into the final version of the RUDAS-PE (**Supplemental Material 1**).

Pilot study (RUDAS in a healthy illiterate elderly population)

Convenience sampling was used to select a group of 30 cognitively healthy illiterate adults (average age 69; no more than 01 year of schooling) from a local senior center. Literacy was self-reported ("Are you able to read or write?"). Cognitive health was based on a standardized neuropsychological evaluation. This study allowed us to verify the validity of the content and criteria.

Mini-Mental State Examination (MMSE)

The MMSE is a BCT that that briefly evaluates cognitive function via 5 main sections: orientation, registration, attention and calculation, recall, and language. A Peruvian version was adapted and validated (modified from the Argentinian version) (16) incorporating cultural modifications specific to Peru. Subsequent studies in Peruvian seniors where most participants were illiterate showed a low sensitivity of 64.1%, a specificity of 84.1%, and a high proportion of false positives (15.9%) (17) indicating that the MMSE is not a good screening test for any type of dementias in geriatric populations.

INECO Frontal Screening Test

The IFS test evaluates executive functions taking ∼10 min to conduct. Its maximum score is 30 points (8 subtests): motor programming (3 points), motor inhibitory control (3 points), backward digital span (6 points), verbal working memory (2 points), spatial working memory (4 points), abstraction capacity (3 points), and verbal inhibitory control (6 points). A Peruvian version of the IFS showed a sensitivity of 94.12% and specificity of 94.2% (18).

Gold Standard

The neuropsychological evaluation that confirmed the cognitive state of the study groups (no-CI, MCI, and dementia) consisted of a battery of tests adapted for use in the Peruvian population. The decision criteria to determine cognitive impairment were two standard deviations less than the average. The neuropsychological battery included the following: DSM-5 criteria, the PFAQ, and CDR.

Diagnostic and Statistical Manual of Mental Disorders (DSM-5)

In the current edition (5th) of the DSM, the diagnostic criteria for neurocognitive disorders (NCD) moves away from the current concept of dementia and MCI taking into account all causes of cognitive impairment irrespective of age group. It is comprised of delirium and two syndromes: major NCD (representing dementia) and minor NCD (representing MCI stage), depending on functionality.

Pfeffer Functional Activities Questionnaire (PFAQ)

The PFAQ is a test that includes 11 questions about daily activities involving money management, shopping, heating water, preparing a meal, staying up-to-date on current events, discussing TV/radio/newspapers, remembering appointments and medication, and traveling outside the neighborhood. Scoring rages from 0 to 3 according to severity of disability in each activity. The maximum score is 33, where a cutoff of seven indicates impaired function (19).

Clinical Dementia Rating (CDR) Scale

The CDR is a global assessment tool (often referred to as global CDR) that was first introduced in the early 1980's to evaluate mild senile dementia of AD (20) and is currently used to measure social changes, behaviors, and functions of the patient. The score is designed to stage dementia severity and is based on independently semi-structured interviews of patients

and informants as well as clinical judgment from the treating physician. It is calculated based on 6 cognitive and behavioral domains including memory, orientation, judgment and problem solving, community affairs, home and hobbies performance, and personal care. It has many advantages: it is independent of other psychometric tests, it does not need a baseline evaluation, it can be used as a control for each individual. Moreover, it has good inter-rater reliability, concurrent validity, predictive validity, and clinical-neuropathological correlation in AD. Its disadvantages include special training requirements, the right skills and good judgment of the interviewer to obtain the pertinent information, and the length of time it takes to be administered (at least 30 min).

Methodological Definition of Illiteracy/Illiterates

A person ≥ 60 years was identified as illiterate by:

1. Determining the years of education attained by asking the patient, "How many years did you go to school?"
2. Asking those with <1 year of formal schooling "Are you able to read or write?"
3. Having those who stated that they could write or read a few words (name and place of birth), confirm they could read a simple phrase.

Participants were selected if they had 0 to $<$ 1 year of schooling and did not know how to read nor write.

Medical Protocol

Random sampling was used to select participants. Subject assent to participate was registered using participant's digital fingerprint and a signature of informed consent from the caregiver/informant, having already been reviewed and approved by the proper regulatory authorities.

Clinical Evaluation

A trained interviewer conducted the clinical evaluation. These procedures included the following: (1) demographic information (via interview and standardized neurologic examination found in the case report forms, (2) anthropometric measures and blood pressure, and (3) comorbidity data and medical treatment received 1 week prior to evaluation.

BCTs and Parametric Test Measurements

Cognitive decline was evaluated in three successive phases: (1) screening - to detect cases with cognitive decline; (2) nosological diagnosis - to determine the specific cause of cognitive decline; (3) final classification of the subjects into their respective group according to their clinical state: controls, MCI, and dementia.

Screening Phase

Field evaluators conducted a clinical neurologic assessment that included anthropometric measurements and blood pressure. Medications taken a week before were recorded as well as responses from their respective caregivers/informants to a subjective memory complaint questionnaire (SMCQ) (questionnaire of memory deficits of everyday life). The PFAQ and BCTs (MMSE, IFS and RUDAS-PE) were administered for the first time.

The cut-off points for this study protocol were as follows: MMSE $<$ 22 for those with 1 to 3 years of education and MMSE $<$ 18 for illiterates; PFAQ $>$ 7.

Nosological Diagnosis: Parametric Tests

All study participants were evaluated twice with <5-week interval between assessments. This time interval (mean 37 $\pm$ days) was defined to yield a higher reliability coefficient. Whenever a BCT was positive for cognitive decline during the screening phase, it was repeated by a different evaluator (neurologist or geriatrician) in the diagnostic phase. Confirmed cases were then identified as patient with cognitive impairment (PCI). In this phase, additional parametric tests were administered to rule-out cognitive impairment from neurodegenerative causes including the Hachinski modified ischemic scale questionnaire, the BDI-II and subsequent RUDAS-PE, MMSE, IFS, and PFAQ tests.

Final Classification: Parametric Tests

The CDR scale was applied by a panel of two evaluators specializing in neuro-rehabilitation and neuropsychology each of whom were blinded to each other's clinical assessments. Next we applied the DSM-5 criteria for major and minor NCDs (corresponding to our study definitions of dementia and MCI, respectively) and the CDR assessment to help determine which stage of dementia the participants were experiencing. The CDR analysis was based on a scale of 0–2: controls (CDR = 0); MCI (CDR = 0.5), early stage dementia (CDR = 1), and moderate stage dementia (CDR = 2). CDR score was applied to both participants as well as to their caregivers/informants. In cases where the assignment of CDR for dementia staging was questionable, a panel consisting of neurologists, geriatricians, neuro-rehabilitators, and neuropsychologists would reach a consensus. Participants who did not present subjective memory complaints on the SMCQ about daily life and also had normal results on all BCTs were considered cognitively healthy and became part of the control group. Evaluators were blinded to a structured neuropsychological evaluation in the third phase. RUDAS-PE results did not form part of the neuropsychological battery used to diagnose and classify subjects into their respective study groups: control, MCI, and dementia.

The evaluation team of the second and third phase (neurologists and neuropsychologist with advanced training in dementia research) were different from the team in the first phase (geriatric residents, psychology and neuroscience students under supervision by a neurologist and medical rehabilitators – also experts in dementia). Throughout the study, experts that applied the neuropsychological tests (gold standard) were blind to the BCTs results.

Data Analysis

Stata version 2.0 (StataCorp LLC, College Station, Texas) was used for data analysis. A descriptive statistical analysis was performed on the demographic and clinical characteristics of the study population as well as on the psychometric properties of the BCTs. $P < 0.05$ were considered statistically significant.

Demographic Characteristics

We applied two-tailed *t*-tests (discrete variables) and Chi Square test (categorical variables) for between-group comparisons.

Psychometric Properties

Reliability

Reliability was tested during the diagnostic phase. Cronbach's alpha coefficient was used to calculate homogeneity and internal consistency. We removed subsequent domains of the RUDAS-PE to evaluate the changes in the coefficient. Lin's concordance correlation coefficient (CCC) and Bland-Altman plots were used to assess test-retest reliability of the RUDAS scores administered to the same population during the first and second phase; the time interval between the two evaluations was <5 weeks. Lastly, inter-rater reliability was also calculated.

Construct Validity

An expert panel of judges consisting of four dementia experts (neurologist SC and DL; neuropsychologists JC and MS) experienced in conducting cognitive and neuropsychological assessments examined the content validity of constructs. A content validity questionnaire (**Supplemental Material 2**) assessed construct-item match and language group suitability.

Criterion-Related Validity: Concurrent, Convergent, and Discriminant

During the second phase, given the non-normally distributed data, concurrent validity was assessed by determining Spearman's rank correlation coefficient (ρ) to measure the strength of a monotonic relationship between paired data: RUDAS-PE/MMSE, RUDAS-PE/IFS, RUDAS-PE/PFAQ, and the RUDAS-PE/CDR, namely for the total RUDAS scores and its cognitive domains in each of these paired test comparisons.

The following Spearman's correlation classification was used:

- 0.0–0.25 "very weak"
- 0.26–50 "weak"
- 0.51–0.75 "moderate to strong"
- 0.76–1.0 "very strong to perfect".

We used logistic regression (logit) for each of the three study group pairs (dementia in early stages/MCI, MCI/control, and dementia in early stages/control) using a two-variable model: final diagnosis as dependent variable, and each BCT as independent variable. For discriminant validity we measured the average of the sum score of the RUDAS-PE and the average score for each of its domains in each of the three groups (controls, MCI, and dementia). These were then compared using the Independent Samples *t*-test. We also analyzed the percentage of individuals correctly classified and conducted a multivariate analysis of variance (MANOVA).

Diagnostic Accuracy

Diagnostic accuracy was evaluated via a post-estimation analysis to configure the Receiver Operating Characteristic (ROC) curves including calculation of the area under the ROC curve (AUC). The maximum values were used to establish sensitivity, specificity, and predictive values. Finally, we compared the AUCs of these BCTs using the Handley and McNeil method.

RESULTS

Participants

The study began with 344 participants; 79 of whom were lost to follow-up: BCTs identified participants with severe stages of dementia ($n = 25$), difficulty attending scheduled visits ($n = 22$), hearing problems ($n = 12$), withdrew informed consent ($n = 11$), and visual problems ($n = 9$). In the second phase, 43 of the 265 participants were excluded: met BDI-II criteria for depression ($n = 21$), absent caregiver/informant ($n = 10$), withdrew informed consent ($n = 7$), and medical reasons ($n = 5$). Thus, 222 participants completed the second phase. An additional 35 participants were excluded in the current analysis for the following reasons: incomplete CDR interview due to absent caregiver ($n = 15$), incomplete CDR interview due to poor collaboration among study participants ($n = 12$), incomplete DSM-5 diagnostic criteria questionnaires ($n = 2$), severe stage dementia ($n = 3$), and moderate traumatic brain injury ($n = 3$) (**Figure 1**).

Clinical and Demographic Profiles

A little over half (56.15%) of the study sample were women. The proportion of females was similar for each study group: dementia (55.6%), MCI (56.7%), and (56.2%) control; there was no significant difference found between study groups. The average age was 70.14 ± 3.79; the control group was significantly younger than the dementia group ($p = 0.000$). Likewise, the MCI group was significantly younger than the dementia group ($p = 0.000$); there were no significant differences in age between the control and MCI groups ($p = 0.794$). All three BCTs (MMSE, IFS, and RUDAS-PE) showed less performance in the dementia group as compared to MCI and control groups. Similarly, MCI patients performed less than the controls. The RUDAS-PE score for the dementia group was 14.97 ± 2.21, 20.43 ± 1.39 for MC,I and 23.87 ± 0.93 for controls. The BDI-II score for the sample population was 6.46 ± 2.98. The BDI-II score in the dementia group was 7.24 ± 3.06, 6.20 ± 2.94 for MCI, and 5.94 ± 2.82 for controls (**Table 1**). None of the groups met the criteria for depression; there was no significant difference between each of the groups based on BDI-II score.

Psychometric Properties of the RUDAS-PE

Internal Consistency

Internal consistency was calculated among all 187 participants completing the third phase. Cronbach's alpha for the RUDAS-PE in a geriatric illiterate population was 0.65. When a RUDAS-PE dominion was removed, the Cronbach alpha coefficient did not increase, on the con trary, the value decreased. For this reason, all the dominions showed to positively contribute to the RUDAS-PE and were consistent throughout the test.

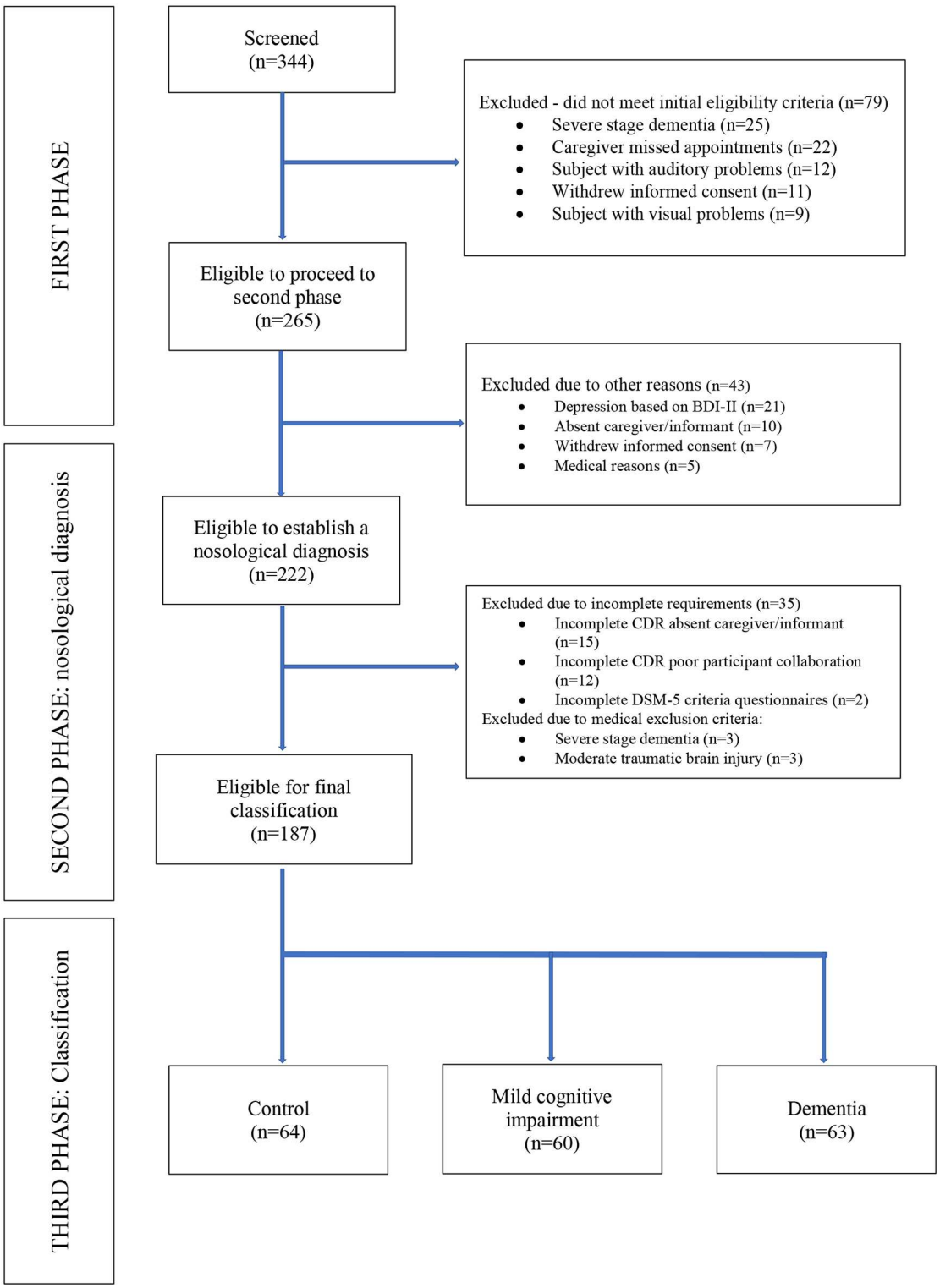

FIGURE 1 | Study flow diagram. Centros de Salud de Ventanilla, Callao, Lima. 2018-2019.

TABLE 1 | Demographic characteristics and brief cognitive test performance according to study groups.

	Control ($n = 64$)	MCI ($n = 60$)	Dementia ($n = 63$)	*p*-value 1 (control vs. MCI)	*p*-value 2 (MCI vs. Dem)	*p*-value 3 (control vs. Dem)
Female (%)	36 (56.2)	34 (56.7)	35 (55.6)	0.554	0.523	0.540
Age in years, mean (SD)	68.92 (3.45)	68.77 (3.14)	72.69 (3.42)	0.794	0.000**	0.000**
MMSE score, mean (SD)	20.16 (1.49)	17.85 (1.64)	10.11 (1.58)	0.000**	0.000**	0.000**
IFS score, mean (SD)	24.06 (1.11)	19.9 (1.34)	14.25 (1.96)	0.000**	0.000**	0.000**
RUDAS-PE score, mean (SD)	23.87 (0.93)	20.43 (1,39)	14.97 (2.21)	0.000**	0.000**	0.000**
BDI-II Score, mean (SD)	5.94 (2.82)	6.20 (2.94)	7.24 (3.06)	0.613	0.058	0.014*

Centros de Salud de Ventanilla, Callao, Lima. 2018-2019.

*BCTs, brief cognitive tests; MCI, Mild Cognitive Impairment; Dem, Dementia; SD, standard deviation; MMSE, Mini Mental State Examination; IFS, INECO Frontal Screening; RUDAS-PE, Rowland Universal Dementia Assessment Scale, Peruvian version; BDI-II, Beck Depression Inventory-second edition. *$p < 0.05$; **$p < 0.001$.*

TABLE 2 | Test-Retest Reliability of the Rowland Universal Dementia Assessment Scale (RUDAS-PE).

Cognitive domain	Phase 1 (test)		Phase 2 (retest)		ρ_c (95% CI)	Difference	Bland and altman limits (95% CI)
	Avg	SD	Avg	SD			
RUDAS-PE total score	20.08	3.42	19.76	3.78	0.61 (0.52–67)	−0.32	−8.59–9.21
Memory	5.24	2.27	5.08	2.11	0.21 (0.12–0.30)	−0.16	−2.84–2.54
Visuo-spatial orientation	4.79	0.62	4.56	0.50	0.54 (0.46–0.61)	0.23	-3.21–3.69
Motor praxis	1.85	0.59	1.66	0.48	0.28 (0.18–0.37)	−0.19	−2.15–1.76
Visuo-spatial construction	0.53	0.56	0.69	0.64	0.25 (0.15–0.33)	0.16	−2.12–1.05
Judgment	1.75	0.67	1.60	0.88	0.44 (0.36–0.51)	−0.15	−2.27–2.06
Language	6.54	1.13	6.92	0.98	0,24 (0.15–0.36)	0.38	−2.92–3.12

Centros de Salud de Ventanilla, Callao, Lima. 2018-2019.

RUDAS-PE, Rowland Universal Dementia Assessment Scale, Peruvian version; SD, standard deviation; Avg, average; CI, confidence interval; ρ_c, Lin's concordance correlation coefficient.

Test-Retest Reliability

Lin's concordance correlation coefficient was used to evaluate the test-retest reliability of the RUDAS-PE based on the scores obtained during the first and second phase of the study (**Table 2**). The total average scores of the RUDAS-PE in the two times that the test was administered were similar (20.08 vs. 19.76); the differences between the two were close to zero (0.32). Meanwhile, Bland-Altman plots showed that the mean differences of the test and re-test included zero for the RUDAS-PE, indicating no significant difference between the two measurements. On the other hand, CCC showed a moderate positive correlation (0.61) between the two observations. Similar patterns were recorded for each dominion of the RUDAS-PE, indicating overall acceptable test-retest reliability.

Intraclass correlation coefficients (ICCs) were also used to assess test-retest reliability resulting in an ICC of 0.96. The correlation between first and second evaluation was 95.9%. The correlation for the RUDAS-PE total score for every dominion were above 40%.

Criterion-Related Validity: Concurrent, Convergent, and Discriminant

Strong correlations were found between the RUDAS-PE/MMSE ($\rho = 0.86$; SD: 0.14, CI 95%), RUDAS-PE/IFS ($\rho = 0.87$; SD: 0.09, CI 95%), RUDAS-PE/PFAQ ($\rho = 0.83$; SD: 0.27, CI 95%), and RUDAS-PE/CDR ($\rho = 0.86$; SD: 0.18, CI 95%).

Discriminant Validity

There was no overlap in the RUDAS-PE scores as depicted in the dispersion graph (**Figure 2**), indicating a very good ability to discriminate between dementia, MCI, and healthy controls. For each BCT, an AUC-ROC curve was calculated for each of the following study groups: (1) control vs. MCI ($n = 124$), (2) control vs. dementia ($n = 127$), and (3) MCI vs. dementia ($n = 123$). The comparison results in the control group and the dementia group showed each of the tests (RUDAS-PE, IFS, and MMSE) approaching an AUC of 1. Similarly, comparing the RUDAS-PE, IFS, and MMSE between the controls and MCI showed an AUC of 1. **Figure 2** shows the ROC curve for the RUDAS-PE (AUC = 0.9828) compared against MMSE (AUC = 0.9999) to discriminate between MCI and controls; meanwhile, **Figure 3** shows the ROC curve of the RUDAS-PE (AUC = 0.9828) compared against IFS (AUC = 0.9959) to discriminate against MCI and controls, where in both cases, the AUC of the RUDAS-PE performed slightly less than the MMSE and IFS, respectively. **Figure 4** shows the differential distribution of the RUDAS-PE according to the scores for each of the diagnostic groups.

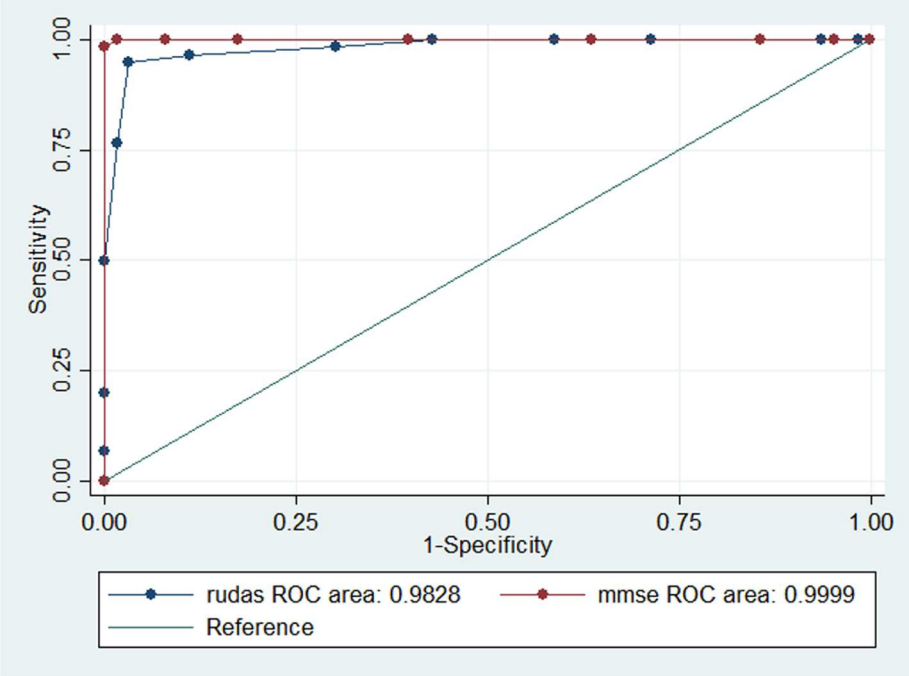

FIGURE 2 | Receiver-operating characteristic (ROC) curve for the Peruvian version of the Rowland Universal Dementia Assessment Scale (RUDAS-PE) and the Mini Mental State Examination (MMSE) in 124 patients for discrimination between mild cognitive impairment (MCI) and control groups. Centros de Salud de Ventanilla, Callao, Lima. 2018-2019.

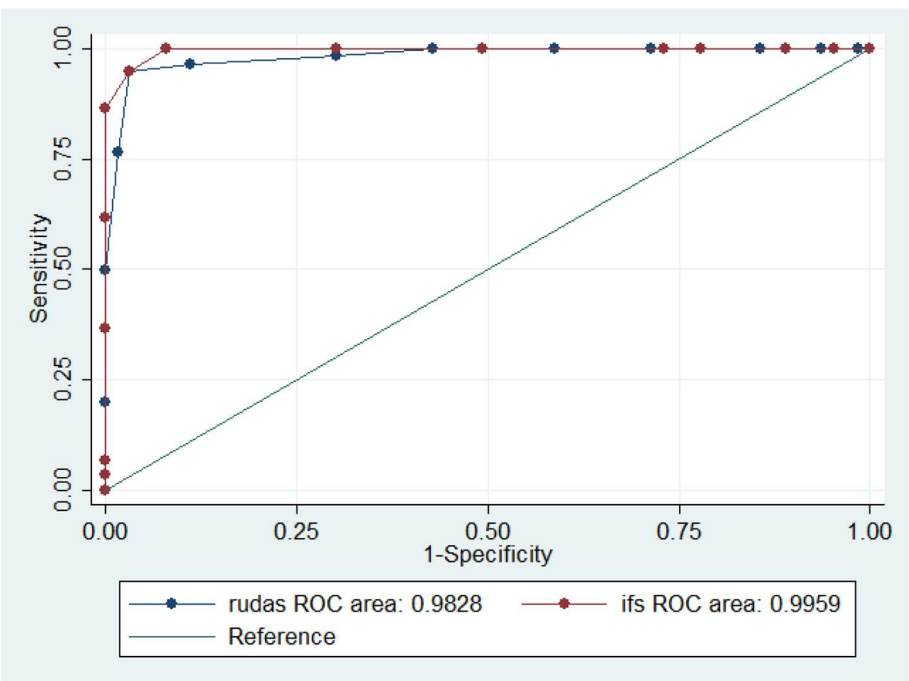

FIGURE 3 | Receiver-operating characteristic (ROC) curve of the Peruvian version of the Rowland Universal Dementia Assessment Scale (RUDAS-PE) and the INECO Frontal Screening (IFS) in 124 patients for discrimination between mild cognitive impairment (MCI) and control groups. Centros de Salud de Ventanilla, Callao, Lima. 2018-2019.

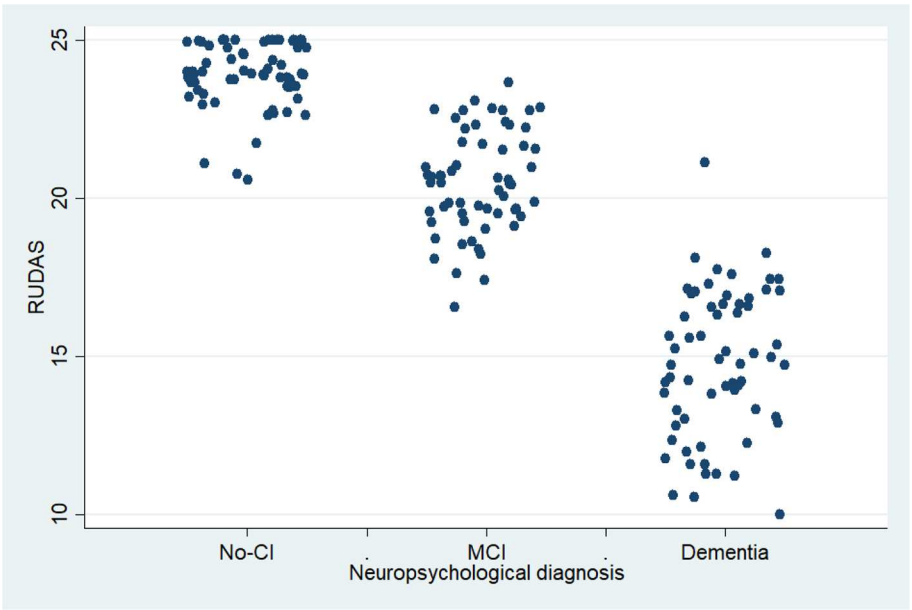

FIGURE 4 | Scores distribution according to neuropsychological diagnosis for the Peruvian version of the Rowland Universal Dementia Assessment Scale (RUDAS-PE), ($n = 187$). Centros de Salud de Ventanilla, Callao, Lima. 2018-2019.

TABLE 3 | Cut-off points and diagnostic performance for the Peruvian version of the Rowland Universal Dementia Assessment Scale (RUDAS-PE), INECO Frontal Screening (IFS) and Mini Mental State Examination (MMSE) to discriminate between controls and patients with mild cognitive impairment (MCI) and dementia.

Diagnostic performance	Discrimination between controls and patients with MCI			Discrimination between patients with MCI and dementia		
	RUDAS-PE	IFS	MMSE	RUDAS-PE	IFS	MMSE
Optimal cutoff point	23	22	19	19	18	14
Sensitivity, %	89.06	100	87.50	95.00	95.00	100
Specificity, %	93.33	93.3	65.00	96.83	96.83	98.41
Youden Index	0.82	0.93	0.53	0.92	0.92	0.98
Correctly classified, %	91.13	96.77	76.61	95.93	95.93	99.19
Likelihood ratio +	13.35	15.00	4.92	29.93	29.93	63.00
Likelihood ratio −	0.18	0.00	0.40	0.05	0.05	0.00
AUC (95% CI)	0.98 (0.96–1.00)	0.99 (0.98–1.00)	0.85 (0.79–0.91)	0.98 (0.96–1.00)	1.00 (0.99–1.00)	1.00 (0.99–1.00)

Centros de Salud de Ventanilla, Callao, Lima. 2018-2019.
MCI, mild cognitive impairment; RUDAS-PE, Rowland Universal Dementia Assessment Scale, Peruvian version; IFS, INECO Frontal Screening; MMSE, Mini Mental State Examination; AUC, area under the curve; CI, confidence interval.

Diagnostic Accuracy

The ability of the RUDAS-PE to discriminate between controls and patients with MCI (AUC: 0.98, 95%CI: 0.96–1.00) was slightly less than the IFS (0.99, 95% CI: 0.98–1.00), however there was no significant difference between both groups ($p = 0.232$). The ability of the RUDAS-PE to correctly discriminate between controls and patients with MCI (AUC: 0.98, 95% CI: 0.96–1.00) was statistically superior to the MMSE (0.85, 95% CI: 0.79–0.91), ($p < 0.05$). On the other hand, to discriminate between patients with MCI and dementia, the AUC of the IFS (1.00 95%CI: 0.99–1.00) and the MMSE (1.00, 95% CI: 0.99–1.00) were similar; both were slightly superior to the RUDAS-PE (0.98, 95% CI: 0.96–1.00), without significant difference ($p = 0.312$) (**Table 3**).

The Youden Index was used to help derive optimal cutoffs to differentiate controls from MCI patients. A score of 23 was selected for the RUDAS-PE (sensitivity 89%, specificity 93%), and 22 for the IFS (sensitivity 100%, specificity of 93%), whereas 19 was the optimal cutoff for the MMSE with an acceptable sensitivity (87.5%) but high proportion of false positives (35%). At the same time, having a compatible MMSE for MCI, generates a small increase in the LR+ (4.92), and a minor decrease in LR- (0.40). Meanwhile in discriminating patients with MCI from dementia, the optimal cutoff was 19 for the RUDAS-PE (sensitivity 95%, specificity 96.83%); 18 for the IFS (sensitivity 95%, 96.83%); and 14 for the MMSE (sensitivity 100%, specificity 98.41%).

TABLE 4 | Comparison of the RUDAS-PE cut-off scores for screening dementia in illiterate and literate populations.

	Control vs. MCI	MCI vs. Dementia
RUDAS-PE illiterate/low-education	23	19
RUDAS-PE literate/mid-education	24	21
MMSE illiterate/low-education	19	14
MMSE literate/mid-education	25	19

DISCUSSION

We have managed to validate the RUDAS from an entire sample of illiterate patients in an urban community; to date, there are only two RUDAS validation studies that include at least half of the illiterate population (13, 14, 21, 22). While another study conducted in Rio de Janeiro (23) included only 10% of illiterates in the AD group and 25.8% in the control group.

The internal consistency of the RUDAS-PE (Cronbach's alpha = 0.65) is in line with previous findings ranging from 0.54 to 0.80 (23, 24). The Spearman's correlation values highlight the usefulness of the RUDAS-PE as a significant predictor of cognitive and functional status confirming our initial findings in a population with a mid-level of education (15). We found a much higher value for the correlation between RUDAS-PE/MMSE (ρ = 0.86) with respect to those reported in previous literature indicating the RUDAS and MMSE correlation to fluctuate between 40 and 80% (25–30).

Based on the Youden index, the optimal cut-off point for the RUDAS-PE to discriminate patients with MCI and controls was 23, with better sensitivity (89%) and specificity (93%), percentage of correctly classified (91%) and LR+ (13) with LR- (0.18) as compared to the MMSE therefore supporting the diagnostic accuracy of the RUDAS-PE over the MMSE in discriminating controls from patients with MCI. These findings are similar to published studies comparing controls and patients with dementia (13–15, 21, 23, 25).

It is worth mentioning that the optimal cut-off scores for both the RUDAS-PE and MMSE in patients with illiteracy and low-levels of education vary from the scores found in literate populations with a mid-level of education (**Table 4**) (15). Taken together, these results would seem to suggest that the performance of the RUDAS-PE is influenced by level of education, but less so than the MMSE.

The probable explanations for the superiority of RUDAS-PE over MMSE in discriminating MCI and controls lie in the structure and weight given to the cognitive domains included in both BCTs. RUDAS-PE, unlike the MMSE, involves verbal fluency, visuospatial or body orientation, motor praxis, and judgment. Thus, assessing executive functions (verbal fluency, judgment) and motor praxis gives RUDAS-PE an advantage over the MMSE by being able to detect changes early in MCI (8–10); while alterations in orientation and attention/concentration occur in early or moderate stages of dementia (31, 32). In addition, administering the RUDAS-PE early could detect other types of dementia such as vascular dementia and the variants of fronto-temporal dementia that cannot be detected with the MMSE (33, 34). On the other hand, the weight attributed to the cognitive domains of the MMSE and RUDAS differs. Thus, while the MMSE concentrates its assessment on orientation, attention/concentration, and language the RUDAS gives greater weight to verbal, body orientation, and visuospatial praxis. This allows the RUDAS to detect different types of dementia syndrome (8, 15).

Limitations include sample size and implications for limited generalizability. A second limitation is the lack of longitudinal follow-up of each case in order to accurately establish the diagnoses of each patient group of our study. Thirdly, the diagnosis of dementia was based only on clinical judgment, without evidence of blood tests, brain images or biomarkers; pathological studies of brain samples could not be performed to establish a definitive diagnosis. A fourth limitation is that this study excluded patients from rural populations or populations whose predominant speech was other than Spanish.

In conclusion, the RUDAS-PE is an acceptable cognitive screening tool that has been validated in both illiterate/low-level of education and literate/ mid-level education populations. Our study proves its performance in discriminating controls from MCI to be superior to the MMSE and similar to both IFS and MMSE in discriminating MCI from dementia. Additionally, the RUDAS-PE is neither influenced by age or sex. Another advantage to the RUDAS-PE is its ease of administration, short application time, and minimal use of equipment. This screening tool has the potential to improve the diagnosis of MCI and dementia with diverse etiologies in the primary care setting.

RECOMMENDATIONS

The link between lower educational achievement and socioeconomic disparities in LMICs is well-established. For developing countries like Peru whose demographic trends reflect a rapidly aging population it is imperative to identify and validate BCTs adapted for this group. An additional challenge lies in that the performance of illiterate individuals on neuropsychological tests often resembles that of literate individuals with dementia, which may contribute to misdiagnosis. We believe that our research will serve as a base for future studies on improving the quality of cognitive screening tools for dementia in low-educated settings. We recommend that further research should be undertaken in evaluating the RUDAS-PE in Peruvian populations that are not Spanish speaking, i.e., Quechua, Aymara, and other dialects. On a wider level, research is also needed to determine the performance of BCTs in primary care centers – where the rates of diagnostic errors tend to be highest,

even in high income countries (35). We propose that further research should be undertaken in developing a differential diagnostic flowchart for geriatric populations focusing on cardiovascular and chronic disease risk factors for cognitive impairment including use of BCTs. In sum, our findings indicate the RUDAS-PE to be an appropriate tool for the discrimination of MCI and dementia in an illiterate and low-educated elderly population.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Instituto de Medicina Tropical "Daniel Alcides Carrión" of the "Universidad Nacional Mayor de San Marcos." The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NC devised the project and main conceptual ideas. NC, RM, DL, and EH-P assisted in the study design. RM, KC, WR-G, JC, and CG collected the data. RM, EH-P, and WR-G organized the database. EH-P and TM performed the statistical analysis. NC, RM, and TM wrote the first draft of the manuscript. NC, RM, KC, MP-C, and TM wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00374/full#supplementary-material

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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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AMBAR, an Encouraging Alzheimer's Trial That Raises Questions

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Grifols' recent Alzheimer Management by Albumin Replacement ("AMBAR") study investigated the effects of plasmapheresis with albumin replacement, plus intravenous immunoglobulin (IVIG) in some subjects, in patients with mild-to-moderate Alzheimer's disease (AD). AMBAR was a phase IIb trial in the United States and a phase III trial in Europe. There were three treatment groups (plasmapheresis with albumin replacement; plasmapheresis with low dose albumin and IVIG; plasmapheresis with high dose albumin and IVIG) and sham-treated controls. Disease progression in pooled treated patients was 66% less than control subjects based on ADAS-Cog scores ($p = 0.06$) and 52% less based on ADCS-ADL scores ($p = 0.03$). Moderate AD patients had 61% less progression, based on both ADAS-Cog and ADCS-ADL scores, than their sham-treated counterparts (p-values 0.05 and 0.002), and their CDR-Sb scores declined 53% less than their sham-treated counterparts. However, ADAS-Cog and ADCS-ADL scores were not significantly different between actively-treated and sham-treated mild AD patients, although CDR-Sb scores improved vs. baseline for treated mild AD patients. Patients administered both IVIG and albumin had less reduction in brain glucose metabolism than sham-treated patients. Questions raised by these findings include: what mechanism(s) contributed to slowing of disease progression? Is this approach as effective in mild AD as in moderate AD? Must IVIG be included in the protocol? Does age, sex, or ApoE genotype influence treatment response? Does the protocol increase the risk for amyloid-related imaging abnormalities? How long does disease progression remain slowed post-treatment? A further study should allow this approach to be optimized.

Keywords: Abeta, albumin, Alzheimer's, AMBAR, clinical trial, intravenous immunoglobulin, peripheral sink hypothesis, plasma exchange

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INTRODUCTION

The amyloid hypothesis (1) led to efforts to treat Alzheimer's disease (AD) by reducing brain Aβ, including vaccination (2), anti-Aβ antibodies (3–7), Aβ aggregation inhibitors (8), β-secretase inhibitors (9), and γ-secretase modulators (10), and inhibitors (11). The failure of these approaches to slow AD's progression [with the possible exception of anti-Aβ antibody Aducanumab, whose recently released findings are controversial (12)] resulted in increased targeting of tau, the main component of neurofibrillary tangles (NFTs), by vaccination (13), anti-tau antibodies (14), tau aggregation inhibitors (15), and kinase inhibitors (16). Other mechanisms which may contribute to AD's neuropathology including inflammation (17), oxidative stress (18), and excitotoxicity (19) have also been targeted, with negative results except for the N-methyl-d-aspartate receptor

antagonist Memantine HCl. Memantine and cholinesterase inhibitors are the only treatments currently approved by the United States Food and Drug Administration for AD; they provide symptomatic benefits to some patients but are not disease modifiers (20).

This Perspective will discuss the results, significance, possible mechanisms, and questions raised by the recently-completed Alzheimer Management by Albumin Replacement ("AMBAR") study (ClinicalTrials.gov ID: NCT01561053) (21) performed by Grifols (Instituto Grifols, S.A.). AMBAR was registered as a phase IIb study in the United States and a phase III study in Europe. The protocol involved plasma removal and its replacement with therapeutic-grade human albumin, plus supplementation with intravenous immunoglobulin (IVIG) in some patients. The rationale for the study was that lowering plasma Aβ levels by this approach might reduce brain levels of soluble Aβ, as predicted by the "peripheral sink hypothesis" (22, 23), possibly slowing AD's progression.

BACKGROUND

In vitro studies found that albumin inhibited Aβ aggregation (24) and neurotoxicity (25). However, plasma albumin from AD patients is more glycated and nitrotyrosinated than plasma from healthy subjects, reducing its ability to inhibit Aβ aggregation (26). Grifols theorized that replacing AD patients' albumin with therapeutic-grade albumin should overcome this problem. Further, therapeutic-grade albumin should more effectively bind plasma Aβ and sequester it than plasma albumin from AD patients. Albumin may protect neurons by additional mechanisms, including anti-oxidant (27, 28) and anti-inflammatory (29, 30) activities. Because of albumin's anti-Aβ effects, Grifols decided to explore the potential of its human plasma albumin Albutein® (31) for treating AD.

The peripheral sink hypothesis is based on the finding that administration of a monoclonal anti-Aβ antibody to a transgenic mouse AD model lowered brain Aβ, despite apparent failure of the antibody to enter the brain (22, 23). This suggested that lowering plasma albumin might result in reduction of brain Aβ by increasing movement of soluble Aβ from brain into peripheral blood. The hypothesis assumes that soluble Aβ is in equilibrium between brain and peripheral blood. Grifols theorized that because ∼90% of plasma Aβ is bound to albumin (32), replacing AD patients' plasma with Albutein, which does not contain detectable Aβ (33), should decrease plasma Aβ (34). The hypothesis predicted that this would result in increased movement of soluble Aβ out of the brain. Some studies have supported the peripheral sink hypothesis (35–37) but others have not (38–40).

PRELIMINARY STUDIES

In 2005 Grifols performed a pilot study (41) with seven mild-to-moderate AD patients who underwent plasma removal with Albutein replacement twice weekly for 3 weeks with a 6-months follow-up period. No clear patterns were detected for changes in plasma Aβ40 or Aβ42. CSF Aβ40 decreased slightly during plasma exchange with a greater decrease in CSF Aβ42, and both Aβ concentrations returned to near baseline 6 months post-treatment. Mini-Mental State Examination (MMSE) and Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-Cog) scores changed little, while imaging suggested increased hippocampal volume and increased frontal and temporal cortex perfusion. In a 1-year extension of the study, a more sensitive method for measuring plasma Aβ40 and Aβ42 revealed a "sawtooth" pattern: Aβ decreased after each plasma exchange, and returned to baseline before the next procedure. CSF Aβ40 and Aβ42 remained relatively stable during the extension. Grifols concluded from these findings that the approach was feasible to consider for treatment of AD patients.

In 2007 Grifols performed a phase II trial (ClinicalTrials.gov Identifier: NCT00742417) (42, 43) with this approach, involving 19 actively-treated and 20 sham-treated mild-to-moderate AD patients. The treatment group underwent plasma removal with Albutein replacement twice weekly for 3 weeks, then weekly for 6 weeks followed by every 2 weeks for 12 weeks. Control patients underwent simulated procedures so neither patients nor study raters knew patient group assignments. Parameters measured were similar to those in the pilot study, following patients for 6 months. The adjusted (least-squares) mean CSF Aβ42 concentration was "marginally higher" ($p = 0.07$), in the treatment group compared to the control group, after the last plasma exchange compared to the mean baseline value, while the change from baseline in CSF Aβ40 was not significantly different between groups. A sawtooth pattern for plasma Aβ40 and Aβ42 was again found in the treatment group. MMSE and ADAS-Cog scores tended to be higher in the treatment group than in the control group at the end of treatment and follow-up periods but between-group differences were not significant (ADAS-Cog $p = 0.09$ at week 21, MMSE $p = 0.08$ at week 44). Higher scores in the treatment group were found for some tests of language and attention, but worse scores for the Neuropsychiatric Inventory (NPI) (44). The frequency of adverse events was similar between groups.

AMBAR

AMBAR was a multicenter, randomized, double-blind, placebo-controlled study in which patients were treated for 14 months. The study included 496 patients with mild to moderate AD (MMSE scores 18–26), divided among three groups of actively-treated subjects and a sham-treated control group. All actively-treated patients initially underwent removal of 2,500–3,000 mL of plasma ("high-volume" plasma exchange), replaced by the same volume of Albutein 5%, weekly for 6 weeks through a peripheral vein or a central venous catheter placed in the subclavian or jugular vein. This was followed by 12 months of monthly "low-volume" plasma exchange in which 650–880 mL of plasma was removed and replaced with 100 mL of Albutein 20% (20 g Albutein), 100 mL of Albutein 20% plus 200 mL of Grifols' IVIG Flebogamma 5% DIF (10 g Flebogamma) ("low albumin/low IVIG" group), or 200 mL of Albutein 20% (40 g Albutein) plus 400 mL of Flebogamma 5% DIF (20 g Flebogamma) ("high albumin/high IVIG" group). This second stage of plasmapheresis was performed via a peripheral vein. ADAS-Cog

and Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL) scores were measured at baseline, after initial plasmapheresis, at 7, 9, and 12 months of second stage plasmapheresis, and at 14 months after finishing plasmapheresis. Primary outcome measures were changes in ADAS-Cog and ADCS-ADL scores between baseline and endpoint. Secondary outcome measures were changes in cognitive, functional, and behavioral tests, measures of disease progression, and alterations in CSF p-tau, total tau, Aβ40, and Aβ42, plasma Aβ40 and Aβ42, brain structure, and brain glucose metabolism. Statistical analyses of changes vs. sham-treated controls in ADAS-Cog and ADCS-ADL scores were performed on data from pooled treatment subjects and, in pre-specified analyses, from patients with mild AD (MMSE 22–26) and moderate AD (MMSE 18–21).

AMBAR's topline results (45) indicated that treatment groups averaged 50 to 75% less worsening of ADAS-Cog scores and 42 to 70% less worsening of ADCS-ADL scores than control subjects. Pooled data from treated subjects showed that these patients declined, on average, 66% less than control subjects based on ADAS-Cog scores ($p = 0.06$) and 52% less based on ADCS-ADL scores ($p = 0.03$). Analyses of changes from baseline to endpoint in patients with moderate AD found 61% less disease progression, based on both ADAS-Cog and ADCS-ADL scores, than sham-treated moderate AD patients ($p = 0.05$ for ADAS-Cog, 0.002 for ADCS-ADL). Although some slowing of disease progression was also found in the treated patients with mild AD, a similar pattern was unexpectedly seen for sham-treated mild AD patients so the between-group differences in ADAS-Cog and ADCS-ADL scores were not statistically significant.

At the 2019 International Congress on Alzheimer's and Parkinson's (AD/PD) (46, 47) Grifols reported significant differences at endpoint between patients in the high albumin/high IVIG treatment arm and the control subjects in tests of memory, language, processing speed, and quality of life. Actively-treated moderate AD patients performed significantly better than their sham-treated counterparts on tests of memory and quality of life, while mild AD patients performed significantly better than their control counterparts on tests of language and processing speed. A low rate of adverse events was reported, occurring mainly during high-volume plasma exchange. CSF Aβ42 was stable in treated patients while decreasing in sham-treated patients (results for Aβ40 were not shown), while CSF phosphorylated and total tau increased less in treated patients than in controls. At the 2019 Alzheimer's Association International Conference (48) Grifols reported that Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change (ADCS-CGIC) scores had remained stable in all treatment groups, and these patients had declined, on average, 71% less than controls on the Clinical Dementia Rating-Sum of Boxes (CDR-Sb) scale (49, 50). CDR-Sb scores for mild AD patients improved while moderate AD patients' scores declined 53% less than their sham-treated counterparts (51). Final results presented at the 2019 Clinical Trials on Alzheimer's Disease (CTAD) Conference indicated that patients receiving both Flebogamma and Albutein had less reduction in brain glucose metabolism than controls.

DISCUSSION

The results from AMBAR are encouraging, in contrast to the other approaches that have been tried to slow AD's progression. A review of AD trials for the period between 2002 and 2012 concluded that the overall success rate was 0.4% (52). No new drugs have been approved for treatment of AD since 2003, although Namzaric, which combines Memantine and Donepezil, received FDA approval in 2014.

Perhaps the most important question raised by AMBAR's findings is: what mechanism was responsible for slowing disease progression? Identifying this mechanism would provide support for further efforts to slow AD's progression by means of the same mechanism. Among the mechanisms that could have contributed to AMBAR's slowing of disease progression are reductions in neurotoxic Aβ species, tau pathology, neuroinflammation, oxidative stress, microcirculatory deficits, and neurotoxic auto-antibodies. These will be discussed below.

Reduced Aβ

Although both Aβ40 and Aβ42 were measured in CSF, results were reported only for Aβ42 (47, 49), whose concentrations were stable in treated patients while decreasing in control patients. Whether brain levels of Aβ were lowered is unclear. CSF Aβ42 is reduced in AD (53), possibly due to sequestration of Aβ42 as insoluble fibrils (54). Lowering soluble Aβ42 in brain could either increase or decrease CSF Aβ42, depending on its rates of clearance from brain to CSF and from CSF to peripheral blood. A future study should measure CSF levels of Aβ soluble oligomers, which may be Aβ's most neurotoxic conformation (55). An assay for their measurement in CSF was recently reported (56). To determine if plaque counts were lowered, PET Aβ imaging could be performed (57, 58). Post-mortem evaluation of plaques and NFT should also be considered on subjects who pass away during a future study with the AMBAR protocol. Plaque densities are less strongly correlated than NFTs with cognitive loss in AD patients (59, 60), so even if plaque counts decreased relative to sham-treated subjects, this would be unlikely to be the sole mechanism responsible for slowing of disease progression. In the AN1792 Aβ vaccination trial, for example, despite marked reductions in plaque counts found in subsequent post-mortem studies (61, 62), clinical progression was not slowed (2). Finally, it would be worthwhile to determine the incidence of amyloid-related imaging abnormalities (ARIA). ARIA refers to imaging abnormalities (often not associated with symptoms) associated with increased movement of Aβ from brain after treatment with anti-Aβ antibodies (5, 63, 64).

Reduced Tau Pathology

The amyloid hypothesis (1) suggests that tau pathology in AD develops downstream from Aβ deposition; therefore if the AMBAR protocol reduced brain Aβ levels, this could have secondarily decreased tau pathology. Total and phosphorylated tau (p-tau) levels in CSF are increased in AD (65). CSF levels of total and p-tau increased less in AMBAR's plasma exchange-treated patients than in sham-treated patients (47), suggesting that tau pathology may have been reduced. A future study should

examine this issue by PET imaging (66). CSF concentrations of soluble tau oligomers could also be measured (67).

Reduced Inflammation

Chronic systemic inflammation has been associated with increased risk for development (68) and progression (69) of AD. Plasmapheresis removes inflammatory cytokines from peripheral blood (70), so the AMBAR protocol could have reduced systemic inflammation via this mechanism, perhaps decreasing brain inflammation as a consequence. Inflammatory cytokines and chemokines, as well as complement proteins and activation fragments, are readily measured in CSF (71–75), so it would be useful to measure these. Activated microglia (76) and astrocytosis (77) can be imaged in the brain via PET, so these procedures should also be considered.

Reduced Oxidative Stress

Oxidative stress in present in AD and may contribute to its pathogenesis (78). The AMBAR protocol could have directly reduced brain oxidative stress due to the anti-oxidant actions of albumin (79, 80) if CSF levels of albumin were sufficient to exert these effects. Conflicting reports have been published regarding the effects of plasmapheresis on oxidative stress (81–84). This could be examined in a future study by measuring CSF oxidative stress biomarkers such as 8-hydroxy-2'-deoxyguanosine (85), 8-isoprostane (86), protein sulfhydryls (87), and total antioxidant capacity (88).

Reduction of Microcirculatory Deficits

Plasmapheresis with removal of low density lipoproteins is used to treat conditions such as familial hypercholesterolemia and peripheral arterial disease. This improves microcirculation and lowers systemic oxidative stress (81). AMBAR's inclusion criteria included diagnosis of AD based on NINCDS-ADRDA criteria, and imaging showing the absence of cerebrovascular disease [which includes stroke, transient ischemic attack (TIA), subarachnoid hemorrhage, and vascular dementia (89)], so AMBAR's participants likely did not have vascular dementia. However, AMBAR's exclusion criteria did not include lipid profile abnormalities, so improved microcirculation might have contributed to slowing of disease progression in some patients. Correlations between plasma lipid profile components and AD progression could be examined in Grifols' next study.

Removal of Autoimmune Antibodies

Plasmapheresis is used to treat some autoimmune disorders because it removes pathogenic auto-antibodies as well as complement proteins and cytokines from plasma (90, 91). Some investigators have suggested that AD may be an autoimmune disorder (92, 93) although this view is not generally accepted. If autoantibodies do play a role in AD pathogenesis, then their removal may have contributed to AMBAR's slowing of AD progression, although this scenario is considered to be unlikely. In the next study with the AMBAR protocol, the presence and titers of CSF anti-hippocampal antibodies (94) could be compared in pre- and post-treatment CSF samples from both actively-treated and sham-treated subjects.

In addition to these mechanisms, plasma exchange removes many other proteins (42, 95) so the possibility is not ruled out that slowing of AD's progression could have been due to lowering of brain levels of unidentified proteins (96).

Grifols reported a low rate of adverse events in AMBAR, many of which occurred during the initial stage of plasmapheresis, which, for some patients, involved placement and 6-weeks maintenance of a central venous catheter. In the phase II trial, anxiety relating to these catheters was suggested to contribute to worse NPI scores in treated patients than in sham-treated patients (42). The decision whether to perform the initial plasma exchange through a peripheral or central vein was "based on the individual characteristics of the patient" (97). The saw-tooth pattern of plasma Aβ40 and Aβ42 was found for both the "high-volume" and "low-volume" stages of plasma exchange, so a future study should clarify if the high-volume plasma exchange (and central venous catheter) is actually necessary.

It is unclear if AMBAR's protocol is as effective in slowing disease progression in mild AD as in moderate AD; this needs clarification. Changes from baseline to endpoint in ADAS-Cog and ADCS-ADL scores indicated significant slowing of progression in the actively-treated moderate AD patients compared to sham-treated moderate AD patients, but no significant differences were found in these scores between actively-treated and sham-treated mild AD patients; however, CDR-Sb scores were improved for actively-treated vs. sham-treated mild AD patients. Although positive effects were reported for mild AD patients in tests of language and processing speed, these effects were notably absent for tests of memory.

Two of AMBAR's treatment groups included Flebogamma. Disappointing results were obtained with IVIG products in phase II and phase III AD trials (98, 99) so IVIG is no longer being considered for AD monotherapy. AMBAR's most positive results with regard to slowing of disease progression were in the high albumin/high IVIG treatment group (46), and neuroimaging similarly found that less reduction in brain glucose metabolism vs. sham-treated patients was found "particularly in patients receiving both albumin and immunoglobulin" (49). IVIG supplies are limited (100) so the supply of Flebogamma could be insufficient to meet the demand for it if the AMBAR protocol receives regulatory approval and the protocol includes Flebogamma. A further concern with IVIG is that it increases serum viscosity (101), predisposing to thromboemboli, particularly in individuals who are immobile or have vascular disease (102).

Shortages of human albumin have also been reported (103), raising the question of whether recombinant human albumin (rHA) could be substituted for human albumin in AMBAR's protocol. rHA has been reported to have a safety, tolerability, and pharmacokinetic/pharmacodynamic profile similar to human albumin (104).

Additional questions about the treatment approach used in AMBAR which need to be answered include the influence of patient age, sex, and ApoE status on slowing of AD progression, the duration of slowing of cognitive and functional decline once

treatment is stopped, and whether the protocol is feasible in the many AD patients who are medically frail, particularly if maintenance of a central venous catheter is required.

CONCLUSIONS

AMBAR's findings are encouraging, despite the questions they raise. A further study offers Grifols the opportunity to address these issues, and to optimize the protocol.

AUTHOR CONTRIBUTIONS

DL wrote and revised the manuscript.

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REVIEW
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Potential New Approaches for Diagnosis of Alzheimer's Disease and Related Dementias

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Dementia is an umbrella term—caused by a large number of specific diagnoses, including several neurodegenerative disorders. Alzheimer's disease (AD) is now the most common cause of dementia in advanced countries, while dementia due to neurosyphilis was the leading cause a century ago. Many challenges remain for diagnosing dementia definitively. Some of these include variability of early symptoms and overlap with similar disorders, as well as the possibility of combined, or mixed, etiologies in some cases. Newer technologies, including the incorporation of PET neuroimaging and other biomarkers (genomics and proteomics), are being incorporated into revised diagnostic criteria. However, the application of novel diagnostic methods at clinical sites is plagued by many caveats including availability and access. This review surveys new diagnostic methods as well as remaining challenges—for clinical care and clinical research.

Keywords: memory, dementia, diagnostic, novel, clinic, Alzheimer

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INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, is now the 6th leading cause of death in the United States (Centers for Disease Control and Prevention). There are close to 50 million individuals with AD globally, and ∼ 6 million individuals in the United States alone (Alzheimer's Disease International). Dementia is an umbrella term and may be caused by many disorders, including several neurodegenerative diseases. The differential diagnosis of dementia in older individuals typically includes AD, vascular dementia (VaD), dementia with Lewy bodies (DLB), Parkinson's disease dementia (PDD), frontotemporal dementia (FTD), and mixed dementias. Infectious etiologies of cognitive decline include neurosyphilis and HIV, and must be ruled out in the work-up of selected individuals. By definition, dementia requires a decline in more than one cognitive domain—memory, praxis, gnosis, language, visuospatial skills, executive function—and may also be accompanied by one or more behavioral disorders—depression, anxiety, personality changes, hallucinations, and delusions. The word dementia, taken from Latin, means "to take away one's mind," and is accompanied by significant social stigma in all cultures. The Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5, 2013) suggested deleting this demeaning term from our medical vocabulary, and proposed instead *major neurocognitive disorder* in order to minimize stigma and discrimination toward those affected (similar to the discontinuation of the demeaning term *mongoloid* in favor of Down syndrome). However, this attempt has mostly failed and most practitioners and advocacy groups still use the term dementia when referring to Alzheimer's disease and related conditions.

Although the Greeks and Romans were well aware of dementia and associated it with aging, Alzheimer's disease (AD) was first reported in 1906 by the psychiatrist Alois Alzheimer. While at a Frankfurt hospital in Germany, Dr. Alzheimer examined his patient Auguste Deter, a 51 year-old woman, and described her as having progressive sleep and memory disturbances, confusion, paranoia, and aggression. Five years later upon her death Dr. Alzheimer, now in his Munich laboratory, investigated the patient's brain employing new silver-staining histological techniques to report the distinctive amyloid plaques and neurofibrillary tangles that continue to define AD pathologically. "*A peculiar severe disease process of the cerebral cortex*" was the title of Dr. Alzheimer's case report. The significance of these autopsy findings was not fully appreciated for many years, but the first diagnosis of AD was made. Now over a century later, brain autopsy remains the only way to diagnose definite AD (since brain biopsy is *not* a standard clinical practice).

Why do individuals with AD and similar progressive dementias often go undetected and undiagnosed? Diagnostic difficulties may be due in part to the variability of symptoms, some of which are difficult to identify. Also, dementia may be confused with other conditions including delirium, depression, anxiety, sleep disorders, side-effects of prescription and over-the-counter drug, drugs and alcohol abuse, and post-concussive syndrome. In addition, the onset of dementia is gradual and insidious, and patients may deny—or lack awareness of—their cognitive deficits. Moreover, given the global and regional variability in medical practice and cultural norms, consensus criteria for diagnosis remain controversial. Recent efforts in standardization of definitions and normal (vs. abnormal) values have resulted in greater harmonization of best practice. Controversies remain, and include the answer to questions such as: How much memory decline is acceptable and considered "normal aging"?

The diagnosis of a dementing illness is based on clinical signs and symptoms. After a medical history and physical examination, including a neurologic and psychiatric assessment, procedures employed to diagnose dementia may include neuropsychological testing, laboratory tests (blood and other biologic fluids), brain neuroimaging, and genetic testing. Methods for detecting changes in brain function and physiology are positron emission tomography (PET) and single photon emission computed tomography (SPECT) (1), which have been utilized in some clinical trials along with blood biomarkers. In some cases structural and functional magnetic resonance imaging (MRI), and to a lesser extent, computed tomography (CT) may be used as well. Typically, in the US and Canada, family medicine physicians, but more likely neurologists, geriatric psychiatrists, and geriatricians are trained in diagnosing individuals with dementia. A clinical diagnosis is important in order to determine prognosis, clinical management (including guiding appropriate prescription medications), genetic implications for family member, and clinical trial participation.

New recommendations to improve dementia detection and diagnosis were introduced by a committee of experts at the Alzheimer's Association's International Conference in Chicago, IL, USA, 2018. The steering committee known as *The Consortium for Detecting Cognitive Impairment, Including Dementia* (DetectCID; https://www.detectcid.org/), was formed by the NIH with a goal to establish, test, and validate methods for detecting cognitive impairment in the public, including underrepresented populations. The purpose of this review is to survey *novel* methods and discuss potential challenges that clinicians face with regard to dementia diagnosis at clinical sites. Adoption of any novel methodology will be limited by practice standards, federal, state/provincial, and local government regulations, cost, and third-party coverage.

BIOMARKERS

Accumulating data has focused on discovering, evaluating, and validating biomarkers for application in clinical research. The goal in many cases is to provide evidence for earlier diagnostic and prognostic capability. Biomarkers are also employed to confirm and improve on diagnostic accuracy of dementia. In AD, biomarker development and validation has focused primarily on cerebrospinal fluid (CSF) -omics, including proteomics, and PET ligands to detect CNS amyloid beta (Aβ) or tau/tangles—the two pathological hallmarks of AD (2–4). However, the use of CSF and PET biomarkers is limited by their invasiveness and cost, respectively. Other challenges concerning biomarker discovery and validation include collection methods, processing procedures, sample storage, and assay standardization within and across laboratories.

In response to these criticisms and concerns, an international working group (Alzheimer's Precision Medicine Initiative) was formed to review the current state-of-art for blood- based AD biomarkers (2). These would be preferable given that blood tests are more feasible in world-wide settings, are less costly (compared to PET) and less invasive (compared to lumbar puncture for CSF collection). To date, 19 blood-based biomarkers were selected by the working group for additional consideration for AD detection. This working group also outlined a pathway from biomarker identification and development to validation so that academic-industrial partnerships in cooperation with regulatory bodies may co-develop putative blood-based AD biomarkers. Validation of biomarkers should start with assessment in a "black and white" panel study. Samples from patients with a diagnosis of AD would be compared to samples in neurologically healthy controls. This "black and white" study would aim to establish a concordance between the novel biomarker and the standard measure. This would be an attempt to validate the overall accuracy of the biomarker in a known group design. The next step is attempting to replicate the results in a set that more accurately reflects primary care. This second study would implement the technology from the developing laboratory and would involve technology transfer to an existing diagnostic assay that is widely available. The next step is refining the diagnostic algorithm, which would allow a case for potential regulatory approval. Finally, to establish interlaboratory replication, samples would be assayed on the intended equipment for regulatory approval. An optional step is the validation using CSF samples obtained

from the same patients in the first study (2). Big data initiatives based on -omics data (Big Data Research and Development Initiative) analyzes large multidimensional blood- based omics data—allowing stratification of populations into well-defined subgroups (sharing commonalties) that may accelerate progress in biomarker development.

There are many challenges related to the validation of blood based biomarkers for dementia. Of 196 candidate blood based biomarkers, only 19 were prioritized for future consideration by the Alzheimer's Precision Medicine Initiative (2). However, none of the 19 blood based biomarkers were deemed to meet the target product profile. Most biomarker candidates were limited by lack of validation in external cohorts. The lack of external validation may lead to selective reporting and inflated predictive accuracy (5). Close cooperation is needed among academia, industry, and regulators to accelerate development of blood based biomarkers for clinical use. Biomarkers are often identified in academia and commercialization is executed by industry. Collaborations between academia and industry would allow for sharing of product testing, access to clinical data, and clinical endpoints (2).

A/T/N System

Some leading investigators propose that 7 biomarkers may be grouped into 3 categories based on their pathophysiology—the so-called A/T/N system (6) where "A" refers to Aβ/amyloid based markers, "T" to tau/neurofibrillary pathology, and "N" to neurodegenerative or neuronal injury markers. This system uses the three categories and rates each category as either positive or negative. For example, a score could be A+/T+/N-, which would indicate the person is positive for Aβ and tau pathology, but negative for markers of neuronal injury or neurodegeneration.

The 7 biomarkers that are grouped into 3 binary categories include higher Aβ/amyloid deposits measured with PET tracer (7), and low CSF Aβ (8–10). These biomarkers also include tau pathology with greater neurofibrillary tangles in CSF phosphorylated tau and a PET tracer of tau (9, 11). Finally, biomarkers of neurodegeneration or neural injury include total tau in CSF, hypometabolism measured with [18F]-fluorodeoxyglucose ^{18}(FDG)-PET, and atrophy on structural MRI in the hippocampus (12).

Biomarkers exist on a continuous scale from normal to abnormal demarcations to have diagnostic categorization of individuals informative for clinical decision making. These demarcations can be arbitrary and many individuals will have biomarkers close to the demarcations, which is true for most diseases and is not unique to AD. The current biomarker measures are not sensitive to low but perhaps clinically significant levels of early pathology (13, 14). The ± binary distinction is a convenient shorthand to increase communication that is easy to use and understand.

NEUROIMAGING

A variety of neuroimaging modalities have been developed with the goal of detecting dementia earlier along the AD spectrum and discriminating among the dementia differential diagnosis. The Alzheimer's Disease Neuroimaging Initiative (ADNI) (15) connects researchers across the US and Canada to collect, validate, and use pooled data (and samples), including MRI and PET neuroimaging, genetic data (genome-wide association study or GWAS), cognitive testing, CSF -omics, and blood-based biomarkers. Although the leading neuroimaging methods are PET and MRI, other modalities are employed such as computed tomography (CT) (16, 17)]. Although CT is considered less sensitive than MRI for studies in dementia, CT is particularly useful for detecting bone lesions and new hemorrhage. Other advantages of CT over MRI include lower cost, shorter acquisition time, and no contraindication with claustrophobia or implanted metallic devices such as a pacemaker. SPECT, a nuclear imaging technique integrating CT and radioactive tracers, is also used in dementia diagnosis including, for example, differentiation of FTD from Jakob-Creutzfeldt Disease (JCD) (18). Functional MRI (fMRI) is a non-invasive technique that measures brain activity indirectly via changes in blood oxygenation. Functional MRI is useful in assessing integrity of brain networks in prodromal stages of AD, thus detecting mild cognitive impairment (MCI) (19, 20) and in discriminating LBD from AD (21).

TRANSCRANIAL MAGNETIC STIMULATION

Transcranial magnetic stimulation (TMS) is a non-invasive therapeutic approach that uses a changing magnetic field to stimulate underlying nerve cells. TMS is under investigation for the treatment of a variety of neurological disorders, including dementia. Benussi et al. found that paired-pulse TMS distinguishes AD from FTD and healthy controls (HC) (22). In this study (n = 175 enrolled and underwent testing), TMS differentiated FTD (n = 64) from AD (n = 79) with a sensitivity of 91.8% and specificity of 88.6%. The authors propose that the observed difference was based on the activity of different intracortical circuits (i.e., cholinergic, GABAergic, and glutamatergic) in AD vs. FTD patients (both groups with mild disease). In other words, by using different TMS paradigms [short-latency afferent inhibition (SAI), short-interval intracortical inhibition (SICI), and intracortical facilitation (ICF)] one may assess the integrity of cholinergic, GABAergic, and/or glutamatergic cortical circuits. Overall, AD and FTD appeared to differ mainly in SICI-ICF and SAI activity where distinguishing AD and FTD from HC (n = 32), resulted in a diagnostic accuracy of >85%.

TMS has also been used to discriminate between atypical Parkinsonian disorders (APD) and AD. In, Benussi et al. (23), APDs such as dementia with Lewy bodies (DLB; n = 27), progressive supranuclear palsy (PSP; n = 13) and corticobasal syndrome (CBS; n = 12) were compared against AD (n = 63) and healthy controls (HC; n = 39). Similar to the TMS study discussed above, an f intracortical circuit activity using TMS paradigms was examined in these different groups. In this study, an overall diagnostic accuracy of 88.3% was found—with individual diagnostic accuracies as follows; 90.5% for AD, 85.2% for DLB, 76.0% for CBS-PSP, and 94.9% for HCs. Collectively

these data suggest that TMS may be useful as a diagnostic tool to discriminate amongst various forms of dementia and other neurodegenerative disorders.

ELECTROENCEPHALOGRAPHY

Electroencephalographic (EEG) recordings have also been assessed for diagnosing dementia. EEG records electrical activity of cortical neurons and thus indirectly represents underlying brain function. EEG recording abnormalities are found in subcortical dementias, for instance, in DLB and PDD. Similar to other methods, the goal is to achieve earlier diagnosis with EEG, which is also a non-invasive technique. However, unlike PET or MRI scanning, EEG recordings are comparatively inexpensive and widely available at clinical centers. EEG methods are sometimes divided into two approaches. The first is accomplished in the resting state (awake at rest) in the absence of any stimulus. Since the patient is not required to perform a behavioral task, it is more comfortable and less stressful for patients (24). There are four effects of AD that have been reported in repeated studies in resting state EEG (25). There is a slowing of the power spectrum from high frequency (alpha, beta, gamma) to a low frequency in patients with AD (26). The shift from higher frequency to lower frequency is proportional to the progression of AD. There is a reduction of EEG signal complexity in patients with AD, which is likely caused by neuronal death (27). Decreased in synchronization is observed in patients with AD, which is a result of decreased connectivity between brain areas (28, 29). The cause of desynchronization is not well understood, it may emerge from atrophy of neural networks. There are neuromodulatory deficits in AD patients with their cross frequency interaction (30). For example, beta rhythms modulated at a theta rate is more pronounced in controls than in AD patients.

The second approach to EEG studies is conducted when the subject is performing a pre-defined task (task-oriented). This approach of task-oriented EEG studies is not ideal for most people with AD since patients have an increase of anxiety and anger. Therefore, performance of simple behavioral tasks may result in discomfort and inability to complete the task (31). In a study by Fraga et al. (32), EEG was used to discriminate among elderly healthy controls (HC; $n = 27$), MCI ($n = 21$) and AD ($n = 15$). This study used EEG analysis during an executive function task (a working memory task). Significant differences were found and EEG was suggested to be useful for early MCI diagnosis, for improved AD diagnosis, and for assessing the probability of MCI progression to AD.

The N100-P200 is elicited by presentation of a stimulus in the absence of task demands representing sensory processes as well as attention and peaks around 200 ms (33). While the traditional view was that the N100-P200 was mostly unaffected and therefore not a good biomarker of AD, others have shown significantly longer latencies for the N100-P200 in familial AD (34). This indicates basic sensory and attentional processes may be compromised in AD.

Other forms of EEG have been tested for diagnosing dementia including quantitative electroencephalography (qEEG)—a computer-based method independent of traditional visual and subjective clinician's interpretation and based on statistical pattern recognition. Studies to date show a high diagnostic value of qEEG when evaluating subjects with AD, MCI, and other types of dementia. For example in a 2015 study by Engedal et al. (35), qEEGs distinguished AD patients from control subjects with a sensitivity of 84% and a specificity of 81%. The qEEGs also separated patients with LBD or PDD from AD with a sensitivity of 85% and a specificity of 87%. This study used a statistical pattern recognition method to analyze qEEG with a user-friendly score extracted from multiple qEEG features. The user-friendly features of this statistical pattern recognition would allow for translation into the clinical setting. The statistical pattern recognition method poorly separated patients with AD from those with MCI. However, in a more recent study by Hogh and colleagues (36), qEEG was used as a diagnostic tool in MCI ($n = 56$) and AD subjects ($n = 32$) vs. health controls (HC; $n = 41$) across several sites in Denmark, Norway, and Sweden. Since the diagnostic and prognostic abilities in this study were low, it would not be appropriate for translation into a clinical setting. Overall however, the statistical pattern recognition method used in qEEG was superior to traditional EEG analysis. Also, the qEEG method correlated well with CSF AD biomarkers, suggesting an association with AD pathologies.

ELECTROVESTIBULOGRAPHY

Electrovestibulography (EVestG) is a vestibular-based diagnostic test that measures field potential activity recorded in the external ear canal in response to vestibular stimuli (37). The EVestG test is very similar to electrocochleography, but with the acoustic input replaced by a series of mechanically-driven orthogonal tilts accomplished by having the subject sit in a tilt chair (tilts in 2 dimensions—left/right and forward/backward). Recordings are made when the chair is static and also while moving. To date, EVestG methodology has been applied toward diagnosis and discrimination of PDD (Dastgheib et al. *Med Biol Eng Comp*, in press) vs. other neurological disorders, such as schizophrenia, depression, and Meniere's Disease (38–40). Overall, sensitivities and specificities have been typically above 85%. EVestG is more than 95% accurate in PDD diagnosis in patients that were at different stages of the disease (41). EVestG may provide a quick and non-invasive screening tool for PDD. Given the accuracy of PPD and PDD diagnosis, future research using EVestG should be conducted in other neurodegenerative disorders.

CLINICAL DECISION SUPPORT

Clinical support systems include computerized alerts, clinical guidelines, patient data reports, documentation templates, reference information, artificial intelligence (AI), automated historical comparisons, and diagnostic support tools (42–45). In particular, computer-based clinical decision support systems have evolved as a *high tech* tool for the objective evaluation and comparison of data for diagnostic purposes (45, 46). One

example is the PredictND tool (47) that was recently tested in the Amsterdam Dementia Cohort ($n = 504$). In this 10 year study, PredictND was highly accurate in separating several types of dementias from each other (i.e., AD, FTD, DLB, or VaD) and from their respective controls (balanced accuracy 82.3%). In addition to the predicted type of dementia, it also provided a confidence measure for classification. Accuracy was highest for VaD and lowest for DLB. In another recent study (48) across several sites in Europe, PredictND was used to differentiate among groups categorized as subjective cognitive decline [SCD; $n = 252$)], AD ($n = 138$), DLB ($n = 20$), FTD ($n = 34$), and VaD ($n = 23$). In this study, 747 patients completed follow-up visits. Of note, the etiological diagnosis changed in 13% of all cases when using PredictND, but the diagnostic accuracy did not change significantly. However, using the PredictND tool increased clinicians' confidence in their dementia diagnosis, indicating that computer-based support systems may assist with clinical decision making. PredictND uses data from neuropsychological tests, MRI, and CSF tests to classify patients according to the disease state index. Prospective studies have possible limitations. The study design was a tradeoff between retaining clinician's impression of patients and minimizing bias from the first to second session. The time between sessions was longer than intended, which may have affected the result. The follow up time was short, especially the evaluation progression of patients with MCI and SCD. However, this approach draws the clinician to data that are most relevant and removes the need to view tens or hundreds of data points individually.

In addition to the disease state index that PredictND used for diagnosis, others have used data from the ADNI to predict progression from MCI to AD (49). The patients underwent neuropsychological testing, MRI scanning, PET scanning, and CSF analysis. The ADNI analyzed MRI and PET scans in MCI patients using the multivariate technique of independent component analysis (ICA). ICA isolates unique features of biomarkers and potentially reveals patterns underlying the imaging data. ICA was able to predict the progression from MCI to AD (50). Support vector machine is a classification algorithm for pattern classification and predicted the progression of MCI to AD (51). However, even with the diagnostic accuracy mentioned in previous studies, clinical support systems are scarce. There is an absence of clear guidelines from regulatory bodies that impedes acceptance of clinical support systems (52). Developers of clinical support systems and its users should propose guidelines that will standardize clinical support systems. Input from both developers and users may result in more clinicians implementing clinical support systems.

Recently, AI applications have been growing. Deep learning is a type of AI that is sometimes described as simulating human learning approaches. Traditional PET image analysis requires an evaluation by experts trained in nuclear medicine and neuroimaging to make pattern recognition decisions. Therefore, deep learning algorithms theoretically may be used to learn and detect features or patterns in PET scans. In addition, deep learning may potentially help recognize additional patterns that are not as obvious during a human clinical review of scanned images. However, the usefulness of this task remains to be seen. Also, given that traditional PET scan analyses are labor intensive, deep learning algorithms may shorten overall review time. In a recent ^{18}FDG-PET study (53), it was hypothesized that a deep learning algorithm could detect patterns not evident on standard human-based clinical image review. PET images were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. The InceptionV3 architecture deep learning algorithm was trained on 90% of the ADNI data set and tested on the remaining 10%, as well as the independent test set. The deep learning algorithm achieved a 82% specificity and 100% sensitivity. These findings imply that not only can deep learning algorithms predict the final diagnosis of AD with high accuracy and robustness, but they may also reduce overall cost due to shorter review times completed in part by machines. However, deep learning algorithms so far have been mainly utilized for the diagnosis of AD (54). Several technical challenges must be overcome to apply deep learning methods to other forms of dementia (54), neuroimaging datasets need a certain amount of labeling time to train a machine learning system, and various types of noise in the images reduces algorithm accuracy, to name a few. Some experts also state the best most AI systems do is reflect the past history's context for the current sample. Finally, once deep learning algorithms are optimized (85–95%) to match specific types of human thinking, there may be no *wiggle* room left for "original" thought. These and other prognostic tools represent valuable support for clinicians. However, it is important to evaluate and compare performance in a standardized manner (55).

OLFACTION AND TASTE

Humans are capable of growing new nerve cells throughout life in a process called neurogenesis—suggesting a novel treatment strategy for dementia. To date, the two human brain regions that are sites of adult neurogenesis are a subfield of the hippocampus and the olfactory bulb (56). In fact, olfactory disorders may predict pre-dementia and dementia (57). Given this, it was hypothesized that olfaction and neurogenesis may be impaired in those with dementia, and that olfactory disorders may predict the conversion from MCI to AD dementia (57). Presently, no gold standard olfactory test is available for diagnosing or monitoring AD in clinical practice, but efforts have been made for predicting AD and for discriminating dementia diagnoses. For example, Williams et al. (58), found that that olfactory impairment was more pronounced in patients with mild DLB than in those with mild AD. Interestingly, in another study (59) patients with AD may demonstrate an asymmetrical decrement of odor detection sensitivity (left worse than right). In this study, the left-right nostril odor detection test functioned as an inexpensive, sensitive and specific test for probable AD.

The human sense of smell aligns with taste perception, and even vision to some degree (60). More recently (61, 62), taste cognition and taste detection were tested in subjects with suspected dementia. In one such study (62), the hypothesis was tested that the insula is associated with taste cognition in patients with AD ($n = 30$) and VaD ($n = 20$) vs. healthy controls ($n =$

15). Overall, it was concluded that glucose metabolism in the right insula was lower in the low taste cognition cohort and VaD patients with insular lesions showed impaired Taste Cognition Test results. Other recent studies (61) suggest that a failure of CNS taste processing occurs in patients with AD.

VISION

Vision is impaired in dementia—with a variety of demonstrable impairment including contrast sensitivity (63). More recent studies (64, 65) examined whether a retinal examination may predict AD earlier and reveal disease progression (66, 67). Mahajan et al. (64), found ocular changes in AD besides decreased contrast sensitivity and included decreased vision, abnormal pupillary reaction, visual field changes, loss of retinal ganglion cells (and retinal nerve fiber layer), peripapillary atrophy, increased cup– disc ratio, retinal thinning, tortuosity of blood vessels, and the deposition of Aβ in the retina.

Examining color vision is also a potentially useful tool for discriminating different types of dementia. For instance, color vision discriminates AD from DLB (68). In this case, it was concluded that color vision deficits in patients with DLB showed a prevalence similar to the defining core features of DLB (∼80%) and may be supportive of a diagnosis of DLB compared to AD. Other studies (69) found color vision differences when comparing AD to VaD. In this study, the sensitivity/specificity analysis was 80.6% and 87.5% for discriminating AD vs. VaD.

Beta-amyloid deposits are found in the retina of patients with AD and are associated with a narrowed lumina and occlusion (70–73). Retinal photography was able to distinguish patients with AD and non-AD with 100% sensitivity and 84% specificity (74). The amyloid levels detected in the retina were correlated with amyloid levels in the brain via PET scan. An increase of 3.5% in retinal amyloid during a 3.5-months period suggests that retinal imaging could be used for monitoring the response to treatment (64). The retinal amyloid test is a screening tool that could complement currently used tests and potentially be used as part of regular eye exams.

SALIVA

Using saliva samples to diagnose AD has several advantages such as the non-invasive ease of acquisition and low cost. Chertkow et al. (75) used saliva and immunoblot analysis to quantify the phosphorylated tau (p-tau)/total tau (t-tau) ratio at different phosphorylation sites. Hyperphosphorylated tau (indicated by p-tau) is a pathological marker for AD. In this study, samples were obtained from AD, MCI, and FTD patients. With one phosphorylation site, Ser-396, the p-tau/t-tau ratio was significantly increased in patients with AD compared with elderly control subjects. However, the sensitivity and specificity were not sufficiently robust to serve as a standard clinical biomarker. In fact, about one third of the AD group failed to show elevations of salivary tau. Another study with saliva (76) measured salivary acetylcholinesterase (AChE) activity in AD—an enzyme deficient in AD patients. The study examined in 15 AD patients who were taking memantine vs. 15 healthy subjects. AChE activity in saliva in the AD group was indeed lower compared to the control group, but there was no significant difference between groups.

SPEECH

Speech impairment is well-known in AD (77) and other dementias and impairment in verbal communication depends on AD stage (78). Progression of speech impairments vary by individual, but three stages are identified (78). In the first, subjects demonstrate word-finding difficulties. In the intermediate stage, vocabulary and language become weaker. In the advanced stage, subjects provide only limited answers consisting of a few words. Nasrolahzadeh et al. (78), examined speech in AD subjects with the goal of utilizing spontaneous speech for earlier detection. This study focused on analyzing and comparing the quadratic phase coupling of spontaneous speech signals from healthy controls ($n = 30$) vs. AD subjects ($n = 60$) using bi-spectrum and bi-coherence methods. Signal processing methods of this type are statistical methods utilizing non-linear interactions of a continuous spectrum of propagating waves in one dimension. All participants were asked to tell "*graceful personal stories, express their feelings, and converse in a friendly way.*" The results showed that the spontaneous speech signal of those with AD was significantly reduced compared to healthy controls.

In another study (79), speech samples were compared in probable AD subjects ($n = 225$) vs. probable DLB ($n = 67$) subjects. In particular, speech samples were evaluated using the Cognitive Status Examination [COGNISTAT; formerly the Neurobehavioral Cognitive Status Examination (NCSE)], in which exam takers discuss what is happening between two people in a presented picture; however, other domains in addition to language may be tested, such as constructional ability, memory, calculation skills, and executive skills. During this test, subjects were scored (in a team effort by several psychologists) based on whether the subjects described or did not describe the relationship between two people during the speech sample. For instance, an example of the description group was as follows; "*This is a picture of fishing. Someone is calling over. The person fishing does not notice a fish caught on the hook because he dozed off.*" In the no-description group, a typical answer may be "*A person is fishing. A person is performing acrobatics on the bridge.*" In addition, study participants were tested with the Mini-Mental State Examination (MMSE). The results suggest that patients with more severe overall cognitive dysfunction and also male patients are less likely to describe the relationship between two people. Difficulties with picture naming tasks are one of the most frequently reported speech impairments in people with AD (80).

Verbal fluency tests are one of the most widely used measures of speech function in patients with dementia (81). These tasks assess the person's ability to retrieve and produce words relevant for the specific task. Letter fluency records the generation of as many words as possible beginning with a given letter, for example words that begin with the letter S. Category fluency involves the generation of as many words as possible that fall into a specific category, for example tools. Letter and category fluency place

demand on executive functioning since patients must engage in verbal retrieval and recall and inhibit incorrect responses. A meta-analysis of 153 studies with 15,990 AD patients found that AD patients had impaired letter fluency (82). Category fluency declines with the progression of AD (83).

Naming difficulty is another well-documented symptom of AD and it typically occurs early in disease onset (81). The Boston Naming Test (BNT) is a widely used test that comprises 60-items ranging from frequent to infrequent items. The patient is presented with an item and allowed approximately 20 s to verbally identify the item. However, some patients with dementia find the 60-item version difficult to complete due to their limited attention. Therefore, the BNT developed two 30-item versions that significantly correlated to each version and the 60-item version. Differences have been found between patients with MCI and controls (84), and between AD and non-AD individuals (85).

A speech language pathologist can modestly improve communication for people with moderate to severe dementia (86). Speech language therapy may offer some protection against further speech decline. However, future studies should examine this question by measuring speech over a long period of time. Also, caregivers can be trained on the methods used by a speech language pathologist, which would lessen the time the patient must to be in the clinical setting.

NEUROPSYCHOLOGIC TESTING

Detailed comparator studies as well as comprehensive reviews addressing tests used for assessing cognitive status in AD and other dementias are published (87–97). Neuropsychologic tests may be organized by cognitive, functional, or behavioral domains (or their combinations) including activities of daily living (ADLQ), short mental status tests (MMSE, MoCA), brief dementia batteries (RBANS), behavioral symptoms (NPI-Q), clinical ratings (CDR), mood (Beck Depression Inventory II), IQ (Wechsler), executive function (Stroop test), visuoperceptual (drawing a clock), language or calculation (BDAE), and episodic memory (paragraph recall, word-list learning, Rey-Osterreith Complex Figure). When administered as a battery, neuropsychological assessments quantify cognitive impairments and rates of progression. The CANTAB is a touchscreen computer automated neuropsychological test battery, which measures learning and memory. Patients with AD are impaired on the CANTAB test battery as compared to controls (98). Virtual reality is used to measure spatial navigation, which is impaired in people with dementia (99). The CANTAB and virtual reality could be integrated into family practice such that any medical professional could administer the task.

CHALLENGES FOR IMPLEMENTATION AT CLINICAL RESEARCH SITES

The goals of biomarker inclusion in newer diagnostic criteria include making a more accurate diagnosis of dementia. However, multiple barriers to implementation of innovative diagnostic methods and biomarkers limit their clinical application. These barriers include patient access to medical care, feasibility, cost, and third-party coverage.

Many individuals with dementia are *never* diagnosed by clinicians. Diagnostic nihilism stems in part from a widely-held perception that currently available drug treatments for AD are inadequate—that they have minimal, if any, benefits, and that risk-benefit and cost-benefit analyses are negative. This current situation with dementia mimics a long-ago time when clinicians chose not to tell patients if they had terminal cancer. Clinicians should make an accurate dementia diagnosis, or refer to a specialty center as needed. If possible, biomarkers should be added to support a dementia diagnosis—as third-party coverage permits. An accurate diagnosis will determine prognosis, guide clinical care and management, enable a discussion of genetic risk with family members, and raise the possibility of clinical trial participation. Newer diagnostic technologies may be available if an individual screens or enrolls in a clinical study (for example, amyloid and tau PET scans, CSF proteomic analysis of Aβ and tau, and ApoE genetic testing).

The development of newer biomarkers (for example, amyloid PET) has uncovered a 10–20 years prodrome of MCI and AD. This population—cognitively normal but at higher risk for AD—is increasingly targeted for clinical research including prevention trials. The creation of databases composed of at-risk volunteers will aid recruitment for clinical studies. Recent efforts are also building trial-ready cohorts of well-characterized individuals in order to improve clinical trial efficiency and lower the high rates of screen-failure.

A significant challenge to implementation is the validation for use in a family practice setting. Clinical support systems need the guidelines of regulatory bodies and communication between the developers and clinicians to implement systems. There are many steps involved to validate blood based biomarkers including establishing a concordance between the novel biomarker and the standard measure, replication in an external laboratory, refinement, transfer to a commercial platform, and validation in an independent cohort. A similar process would be implemented to validate saliva based biomarkers. The computer based neuropsychological testing with an iPad touchscreen or virtual reality could be administered by any medical professional in the family practice setting.

CONCLUSION

In addition to a traditional medical history and neurologic examination, new technologies may assist in the diagnosis of dementia or impending dementia due to neurodegenerative disorders. Newer iterations of diagnostic criteria are incorporating validated diagnostic biomarkers, when available, as supportive evidence of a particular dementia diagnosis. Controversies remain, however, regarding the optimal biomarker, or combination of biomarkers, to include in these criteria. A lack of consensus of expert opinion, however, is not the only limitation to their clinical application. Operational issues

including availability, feasibility, cost, and third-party coverage will limit their incorporation into clinical practice. Novel diagnostic biomarkers, particularly if relatively inexpensive and non-invasive, have the potential to markedly improve current practice, with added value in screening, prognosis, accurate diagnosis, and evaluation of novel treatments now under development for dementias including dementia due to AD.

AUTHOR CONTRIBUTIONS

RT added a perspective from his memory clinic that he directs. TS added content relevant to CROs. BA who holds 2 dementia research chairs, was responsible for much of the research. DD revised a portion of the manuscript. All authors contributed to the writing of the manuscript.

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CASE REPORT
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Non-fluent/Agrammatic Variant of Primary Progressive Aphasia With Generalized Auditory Agnosia

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Cortical neurodegeneration-induced non-fluent/agrammatic variant of primary progressive aphasia (nfvPPA) is a clinical syndrome characterized by non-fluent speech, such as apraxia of speech or agrammatism. We describe the case of an 80-year-old right-handed woman who exhibited nfvPPA. Atypically, our patient also presented with generalized auditory agnosia. Brain magnetic resonance imaging revealed left-sided predominant atrophy of the bilateral perisylvian area, including the inferior frontal and superior temporal lobes. In a series of auditory tasks assessing generalized auditory agnosia, our patient was unable to accurately identify verbal sounds, environmental sounds, or familiar Japanese songs that she could sing. In the context of recent studies, our study indicates the existence of a clinical syndrome characterized by progressive speech disorder with auditory agnosia. This case report thus provides novel insights into the spectrum of language impairment induced by neurodegenerative disease.

Keywords: agrammatism, amusia, apraxia of speech, environmental sound agnosia, word deafness

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BACKGROUND

Primary progressive aphasia (PPA) is a collective term for neurodegenerative diseases that present with language impairment as the most salient feature. Consensus criteria were proposed in 2011 for three clinical syndromic variants of PPA: non-fluent/agrammatic (nfvPPA), semantic, and logopenic (1). nfvPPA is characterized by non-fluent speech, such as apraxia of speech (AOS) or agrammatism; the semantic variant of PPA, by anomia with loss of the meanings of single words; and the logopenic variant of PPA, by anomia without loss of the meanings of single words, sentence repetition deficits, and phonological errors. However, recent evidence suggests the existence of an additional, atypical variant of PPA (2–4), indicating that the established consensus criteria may not account for the full range of clinical syndromic variants of PPA. Herein, we present the case of a patient with nfvPPA and generalized auditory agnosia to further expand our knowledge of the spectrum of language impairment in neurodegenerative diseases.

CASE PRESENTATION

Case Description

An 80-year-old, right-handed woman visited our hospital because of gradually progressive difficulty in speaking and recognizing spoken words. She had received 9 years of education. Speaking and recognizing spoken words had concurrently become challenging at around the age of 77 years.

Except for the presence of cataract, her medical history was unremarkable. She was fully conscious and oriented at the initial visit. No abnormalities were detected on physical and neurological examinations, or routine laboratory tests. Brain magnetic resonance imaging revealed left-sided predominant atrophy of the bilateral perisylvian area (**Figure 1A**). There was no evidence of hemorrhage or ischemic lesion. *N*-Iso-propyl-*p*-[123I] iodoamphetamine single-photon emission computed tomography (SPECT) revealed predominant left-sided hypoperfusion of the bilateral frontal and temporal lobes (**Figure 1B**). To assess the patterns of hypoperfusion (5), SPECT data were analyzed with 3D stereotactic surface projections (SSP) (6). All SPECT scans underwent realignment, spatial normalization, and non-linear warping. The scans were sampled at 16,000 predefined cortical locations and projected on a 3D image. The voxel values of the patient's SPECT data were normalized to the whole brain's tracer uptake and compared with an age-matched normal database, yielding a 3D SSP Z score image. The abnormalities of cerebral hypoperfusion were displayed with a Z score map. Z scores were calculated using the following equation: Z score = (normal mean − patient mean) / (normal standard deviation). We used a Z score of 2 as the cutoff value in each voxel, and voxels with a Z score ≤ 2 were considered voxels without significantly decreased regional cerebral blood flow. Brain SPECT data analyzed with 3D SSP revealed relative hypoperfusion, mainly in the left superior temporal and inferior frontal gyri (**Figure 1C**).

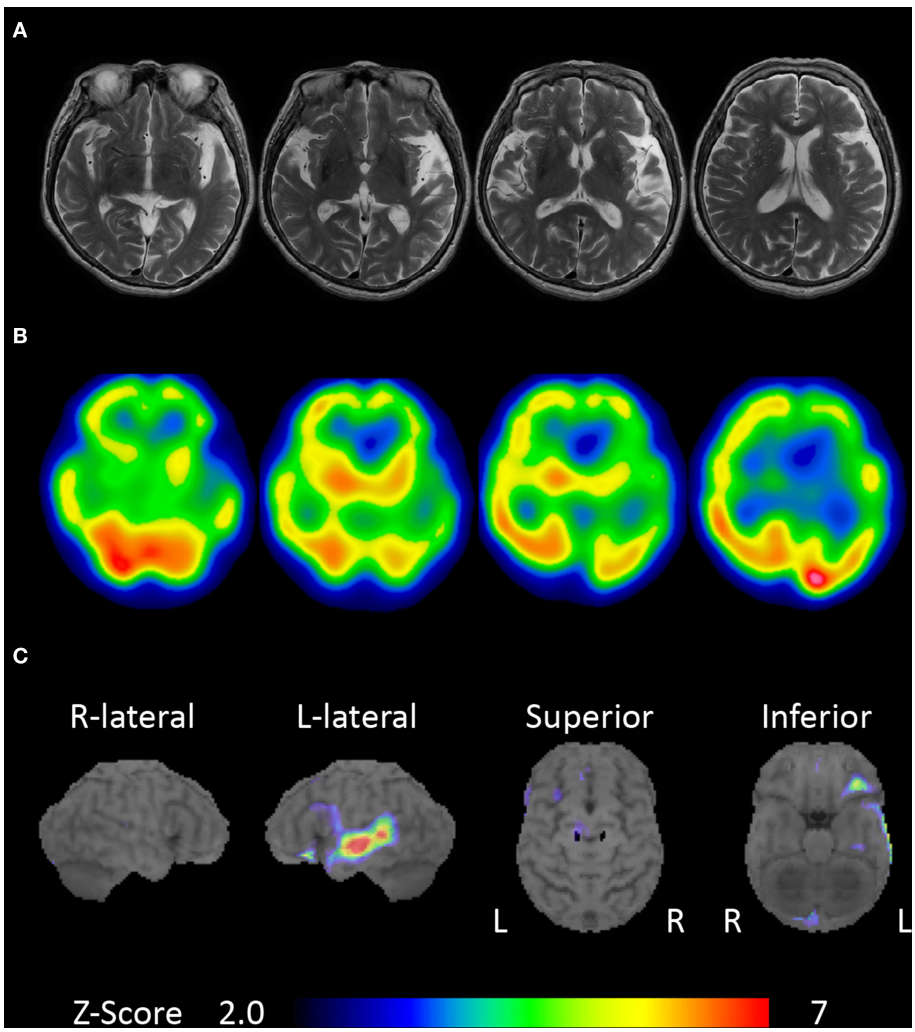

FIGURE 1 | Brain magnetic resonance imaging and single-photon emission computed tomography (SPECT). **(A)** Brain magnetic resonance imaging showing left-sided predominant atrophy of the perisylvian area. **(B)** *N*-Iso-propyl-*p*-[123I] iodoamphetamine SPECT showing left-sided predominant hypoperfusion of the bilateral frontal and temporal lobes. **(C)** Brain SPECT analyzed with 3D stereotactic surface projections (SSPs) showing relative hypoperfusion mainly in the left superior temporal and inferior frontal gyri.

Neuropsychological Examination

Detailed neuropsychological evaluations were performed in the month following the initial visit. Detailed data obtained from standard neuropsychological tests are presented in **Table 1**. The Wechsler Adult Intelligence Scale—Third Edition (WAIS-III) with written instructions (7) revealed a full-scale intelligence quotient (IQ) of 98, verbal IQ of 84, and a performance IQ of 116. Using the Wechsler Memory Scale—Revised with written instructions (7), we found a general memory index of 88, verbal memory index of 80, visual memory index of 107, delayed recall index of 84, and attention/concentration index of 92. These findings indicated that the patient's intelligence and memory were normal.

Her spontaneous speech was shown to be monotonous, slow, and effortful using the Western Aphasia Battery [Japanese edition; (8)]. Her articulation was impaired due to AOS. Connections between syllables were frequently prolonged. She sometimes exhibited distorted sound substitutions and stuttering without self-correction. We occasionally observed telegraphic speech characterized by the omission of grammatical morphemes, which is a component of agrammatism. She experienced difficulty in producing sentences, and her speech was limited mainly to short utterances. She recognized spoken words with difficulty; hence, repetition and auditory comprehension were impaired. Moreover, she could not write words to dictation. However, the Token test with written questions demonstrated that her comprehension of written language was completely preserved (166/166; the mean score in four age-matched healthy controls at our hospital was 164.8 ± 1.5). She could correctly write what she wanted to say in both Kana and Kanji. Except for buccofacial apraxia, praxis was intact. No acalculia was noted.

TABLE 1 | Performance on standard neuropsychological tests.

	Score	Normative data; mean (SD)
WAB		
Aphasia quotient (100)	49.8	97.7 (3.0)
Fluency (10)	5	10.0 (0)
Information content (10)	8	9.7 (0.6)
Auditory comprehension (10)	5.2	9.8 (0.1)
Repetition (10)	0.4	9.9 (0.3)
Naming (10)	6.3	9.5 (0.6)
Reading (10)	9.1	9.5 (0.8)
Writing (10)	7.1	9.6 (1.0)
Praxis (60)	57	59.8 (0.7)
Calculation (24)	24	23.1 (2.3)
Token test		
Auditory comprehension (166)	6	163.6 (2.0)
Reading (166)	166	164.8 (1.5)
WAIS-III		
Full IQ	98	100.0 (15.0)
Verbal IQ	84	100.0 (15.0)
Performance IQ	116	100.0 (15.0)
Raven's colored matrices (36)	32	24.9 (5.3)
WMS-R		
General memory index	88	100.0 (15.0)
Verbal memory index	80	100.0 (15.0)
Visual memory index	107	100.0 (15.0)
Attention/concentration index	92	100.0 (15.0)
Delayed recall index	84	100.0 (15.0)

The maximum score is noted in each row header.
WAB, Western Aphasia Battery; WAIS-III, Wechsler Adult Intelligence Scale—Third Edition; IQ, intelligence quotient; WMS-R, Wechsler Memory Scale—Revised; SD, standard deviation.

Special Assessments for Auditory Agnosia

The following special assessments were administered with written instructions.

Pure Tone Audiometry and Speech Audiometry Test

Slight sensorineural hearing loss was detected (43.8 dB in the right ear and 41.3 dB in the left) with a standard pure tone threshold audiometry test (**Table 2**). On the other hand, a speech audiometry test consisting of monosyllabic sounds showed discrimination of 0% at 10–90 dB for both ears (**Table 2**), although registration of pure tones was mostly preserved. These results revealed that our patient had severe word deafness.

Temporal Auditory Acuity Measures

To examine the temporal resolution of the auditory system, click fusion, and counting tests were performed following the method used by Albert and Bear (9). In the click fusion test, intervals between two brief binaural pulses were varied, and the patient was asked to report whether she heard one or two clicks. Normal controls can distinguish two clicks presented at 1–3-ms intervals (10); however, our patient could not distinguish clicks presented at intervals of 400 ms according to ascending and descending limits (**Table 2**). In the click-counting test, the patient was asked to count the number of clicks presented in 1 s. While the number of clicks countable by normal controls in 1 s ranges from 9 to 11 (11), our patient's count was inaccurate at rates of

TABLE 2 | Performance on auditory tests.

	Score	Normative data; mean (SD)
Pure tone threshold	R 43.8 dB L 41.3 dB	
Speech audiometry	Both 0% (10–90 dB)	
Click fusion	500 ms	1–3 ms
Click counting	2	9–11 counts
Recognition of environmental sounds (20)	7	20.0 (0)
Recognition of familiar Japanese songs (20)	11	20.0 (0)

The maximum score is noted in each row header.
SD, standard deviation.

>2 clicks/s (**Table 2**). These results revealed that the temporal auditory resolution of our patient was severely impaired.

Recognition of Environmental Sounds

We assessed our patient's ability to recognize non-verbal sounds. Twenty environmental audio recordings consisting of the following four sound categories were presented to both ears: human non-verbal (e.g., baby crying), manmade inanimate (e.g., running water), non-human animate (e.g., dog barking), and natural inanimate (e.g., wind) (12, 13). After hearing each sound, she was asked to name the environmental sound. While the mean score of four age-matched healthy controls from our hospital was 18.0 ± 0.7, our patient could name only 2 of the 20 (10%) sounds. After the naming task, the patient was asked to match one of the four pictures to a presented sound (12, 13). The four controls easily identified the correct answers and achieved a common score of 20.0. Our patient provided correct responses for 7 of the 20 (35%) sounds (**Table 2**); as an example, she selected a picture of a vacuum cleaner when the sound of a ringing phone was played. These results revealed that our patient had environmental sound agnosia.

Recognition of Familiar Japanese Songs

The patient was asked to sing 20 familiar Japanese songs without accompaniment but with the provision of the song title and lyrics in writing; a correct response was noted when the patient's singing preserved most of the original melody. The singing score of four age-matched healthy controls from our hospital was 16.0 ± 1.6. Our patient provided correct responses for 17 out of 20 (85%) songs; her memory of the 20 songs thus seemed to have been intact. We then presented each of the songs, and the patient was asked to name the song's title or artist. While the mean naming score of the four healthy controls was 14.0 ± 1.4, our patient could identify the title and artist for only 1 out of the 20 (5%) songs. Finally, each of the 20 songs was presented to the patient, and she was asked to match the album cover, written song title, and artist name with the song in a four-alternative forced-choice paradigm. The controls were easily able to choose the correct answers and achieved a common score of 20.0. Our patient provided correct responses for 11 out of the 20 (55%) songs (**Table 2**). Although her singing of the familiar Japanese songs was well-preserved, she was largely unable to recognize the same Japanese songs after hearing them. These results evinced receptive amusia.

DISCUSSION

Herein, we present a case of unclassifiable PPA: a combination of nfvPPA and generalized auditory agnosia. The patient's speech fluency was impaired due to AOS and agrammatism, the core features of nfvPPA. She did not exhibit any problem with object knowledge as indicated by the WAIS-III score, which further supported a diagnosis of nfvPPA. In addition, neuropsychological examination revealed that she did not exhibit any problems other than conversation. Hence, except for the generalized auditory agnosia, this patient met all the criteria for nfvPPA (1).

The anterior components of the language network, including the inferior frontal lobe, and the anterior opercular and perisylvian areas, including the anterior insula and superior temporal gyrus, have been implicated as the neuroanatomical substrates of nfvPPA (14). The lesions identified in our case correspond to these areas and may therefore account for the observed language impairments, including generalized auditory agnosia, which is seldom observed in typical nfvPPA.

Generalized auditory agnosia refers to a rare impairment in the ability to recognize sounds despite adequate hearing ability, as measured using standard audiometry (15, 16). On the other hand, selective auditory agnosias refer to impairments in the ability to recognize specific categories of sounds. For example, pure-word deafness and non-verbal auditory agnosia of environmental sounds or music are considered to be selective auditory agnosias. Our patient exhibited severe word deafness despite adequate hearing ability. Her impaired temporal auditory acuity, revealed by the click fusion and counting tests, indicated the diagnosis of word deafness, which has been observed in previous patients (9, 11, 17–19). Moreover, our patient discriminated environmental sounds with difficulty and could not recognize familiar Japanese songs, even though her ability to sing those songs was well-preserved. Therefore, these results revealed that our patient had generalized auditory agnosia.

Generalized auditory agnosia, as reported in cases of cerebrovascular disease (20) and neurodegenerative disease (7, 16, 21), is associated with bilateral temporal lobe lesions involving the primary auditory and auditory association cortices; our patient's lesions, identified using brain magnetic resonance imaging and SPECT, correspond to these previously elucidated areas. Moreover, brain SPECT analyzed with 3D SSP revealed relative hypoperfusion mainly in the left superior temporal gyrus, which is consistent with patterns of hypometabolism identified using positron emission tomography with ^{18}F-labeled 2-fluoro-2- deoxyglucose (7). Therefore, in the context of past research, we suspect that generalized auditory agnosia in our case was induced by bilateral temporal lobe atrophy involving the superior temporal gyrus.

Both verbal auditory (word deafness) and non-verbal auditory agnosia have been reported in the stroke literature (20) but are rarely reported in the setting of progressive neurological disorders (7). Recent evidence suggests the existence of a clinical syndrome characterized by progressive speech disorder and auditory agnosia in progressive neurological disorders: Iizuka et al. reported the case of a patient with AOS and word deafness (13); Kaga et al. described a case of AOS, word deafness, and environmental sound agnosia (22); Otsuki et al. observed the concurrent presentation of dysprosody, word deafness, and environmental sound agnosia (18); Ota et al. reported the case of a patient with progressive foreign accent syndrome and word deafness (19); Sakurai et al. described the case of a patient with unclassifiable PPA who exhibited paragrammatism, recurrent utterance, and word deafness (23); and Kuramoto et al. described the case of a patient with unclassifiable PPA with undifferentiated jargon, word deafness, and environmental sound agnosia (24). Furthermore, Utianski

et al. reported the case of a patient with unclassifiable PPA who exhibited phonological errors and agrammatism of spoken and written language on first assessment (5 years after symptom onset) (7); the same patient subsequently exhibited worsening of aphasia and developed AOS as well as verbal auditory (word deafness) and non-verbal auditory agnosias. Furthermore, Mesulam et al. documented the case of a patient with unclassifiable PPA who exhibited agrammatism of spoken and written language as well as profound impaired auditory word comprehension relative to her visual word comprehension (25); the dissociation of her comprehension between auditory and visual word processing was speculatively attributed to auditory word-form area dysfunction because she could discriminate phonemes—i.e., her impairment of auditory word processing level differed from that of our patient. The other cognitive functions of the patients presented in these cases were well-preserved. The patient described by Iizuka et al. (13) subsequently developed behavioral problems. Our patient exhibited speech disorder, aphasia, word deafness, environmental sound agnosia, and receptive amusia. She did not exhibit any other cognitive impairment or behavioral problems. Moreover, recent studies have shown that patients with nfvPPA show deficits of non-linguistic auditory analysis (26, 27). However, we could not find any reports of patients with early-stage neurodegenerative diseases and unclassifiable PPA that exhibited nfvPPA and generalized auditory agnosia. To the best of our knowledge, no prior studies have involved extensive auditory examinations of a patient with nfvPPA and generalized auditory agnosia. The present case report, therefore, suggests the existence of a clinical syndrome characterized by progressive speech disorders and auditory agnosia and provides novel insight into the spectrum of language impairment induced by neurodegenerative disease.

The present study has several limitations. First, although we believed that the auditory agnosia was in the context of language deficits (7), the presence of cognitive impairments (auditory agnosia) other than aphasia may exclude a PPA diagnosis (1). Second, our patient did not undergo additional tests, other than the Western Aphasia Battery, to assess writing ability or further formal evaluation, such as the measurement of auditory-evoked potentials, to assess sensory functioning. Third, we did not perform a cerebrospinal fluid biomarker analysis. Moreover, no pathological findings were obtained in the present case, and therefore, this issue requires further investigation.

CONCLUSION

The current study describes a rare case of unclassifiable PPA: a combination of nfvPPA and generalized auditory agnosia caused by neurodegenerative disease. In extensive auditory examinations assessing generalized auditory agnosia, our patient was unable to accurately identify both speech and non-speech sounds. Our results provide novel insights into the spectrum of language impairment induced by neurodegenerative disease.

DATA AVAILABILITY STATEMENT

All data generated in this study are included in the article/supplementary material.

ETHICS STATEMENT

Written informed consent was obtained from the patient and her family members for publication of this case report and any accompanying images.

AUTHOR CONTRIBUTIONS

HW acquired case data, designed the study, and drafted the manuscript. MI and EM supervised the study and helped to draft the manuscript. All authors contributed to the article and approved the submitted version.

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frontiers in Neurology

CASE REPORT
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Increased Neurofilament Light Chain and YKL-40 CSF Levels in One Japanese IBMPFD Patient With VCP R155C Mutation: A Clinical Case Report With CSF Biomarker Analyses

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Inclusion body myopathy (IBM) with Paget's disease of bone (PDB) and frontotemporal dementia (IBMPFD) presents with multiple symptoms and an unknown etiology. Valosin-containing protein (*VCP*) has been identified as the main causative gene of IBMPFD. However, no studies on neurofilament light chain (NFL) as a cerebrospinal fluid (CSF) marker of axonal neurodegeneration or on YKL-40 as a CSF marker of glial neuroinflammation have been conducted in IBMPFD patients with *VCP* mutations. A 65-year-old man presented with progressive muscle atrophy and weakness of all limbs, non-fluent aphasia, and changes in personality and behavior. Cerebral MRI revealed bilateral frontal and temporal atrophy. ^{99m}Tc-HMDP bone scintigraphy and pelvic CT revealed remodeling changes and active osteoblastic accumulations in the right medial iliac bone. Muscle biopsy demonstrated multiple rimmed vacuoles in muscle cells with myogenic and neurogenic pathological alterations. After the patient was clinically diagnosed with IBMPFD, DNA analysis of the *VCP* gene revealed a cytosine (C) to thymine (T) (C→T) mutation, resulting in an amino acid exchange of arginine to cysteine (p.R155C mutation). The CSF levels of NFL at two time points (12 years apart) were higher than those in non-dementia controls (CTR) and Alzheimer's disease (AD); lower than those in frontotemporal dementia with motor neuron disease (FTD-MND); and comparable to those in patients with behavioral variant frontotemporal dementia (bvFTD), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS). The CSF levels of YKL-40 were comparable at both time points and higher than those in CTR; lower than those in FTD-MND; and comparable to those in bvFTD, PSP, CBS, and AD. The CSF levels of phosphorylated tau 181 (P-Tau) and total tau (T-Tau) were not significantly different from those in CTR and other neurodegenerative diseases, except those in AD, which were significantly elevated. This is the first report that demonstrates increased NFL

and YKL-40 CSF levels in an IBMPFD patient with a *VCP* mutation (p.R155C); NFL and YKL-40 levels were comparable to those in bvFTD, PSP, CBS, and AD and higher than those in CTR. Our results suggest that IBMPFD neuropathology may involve both axonal neurodegeneration and glial neuroinflammation.

Keywords: IBMPFD, *VCP*, mutation, CSF, NFL, YKL-40, AD, frontotemporal dementia

INTRODUCTION

Inclusion body myopathy (IBM) with Paget's disease of bone (PDB) and frontotemporal dementia (IBMPFD) is a multi-organ disease with still unknown etiology (1, 2). In IBMPFD with autosomal dominant inheritance, valosin-containing protein (*VCP*) has been identified as the major causative gene (2, 3). Neurofilament light chain (NFL), which is indicative of axonal neurodegeneration (4), has been validated as a CSF biomarker of behavioral variant frontotemporal dementia (bvFTD), FTD with motor neuron disease (FTD-MND), amyotrophic lateral sclerosis (ALS), progressive supranuclear palsy (PSP), corticobasal syndrome (CBS), and Alzheimer's disease (AD) (5–7). Furthermore, YKL-40 (known as chitinase 3-like 1) has been reported as a CSF biomarker of glial neuroinflammation in neurodegenerative diseases (6–8). With regard to IBMPFD with *VCP* mutations, no CSF studies on NFL or YKL-40 have been conducted yet.

CASE PRESENTATION

We describe the case of a 65-year-old man who presented with muscle weakness and atrophy of all limbs. At the age of 42 years, he experienced difficulties in standing from a sitting position and raising his arms over his head. At the age of 48 years, he was affected by gait disturbances with difficulties squatting and was able to walk only at a slow pace. Further, the patient could not raise his arms over his head and experienced difficulties moving his head and neck freely. These symptoms gradually deteriorated. During the first hospitalization at the age of 52 years, the patient showed atrophy and weakness of the muscles of all limbs but most prominently of the bilateral quadriceps. The neuropsychological examination revealed decline in his cognitive function. The scores of the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) were 26/30 and 18/30, respectively, with disturbances of attention and executive functions. The score of the frontal assessment battery (FAB) was 8/18 with disturbances of "similarities," "lexical fluency," and "motor series." However, no remarkable changes of character, behavior, voice, and speech were observed. The patient showed generalized hyporeflexia without pathologic reflexes. He exhibited no respiratory difficulty. The CT showed prominent atrophy of the quadriceps and other muscles, e.g., hamstrings, iliopsoas, and anterior tibial muscles (not shown). Because of gait difficulty due to weakness of the legs, the patient used a cane or a walker at the age of 52 years (after the first hospitalization), and he used a wheelchair at the age of 55 years. He had occasional cough due to dysphasia and difficulty expectorating, when he was 60 years old; at the same time, he exhibited character changes including self-centered thinking, extreme dependence on his wife, irritation, and frustration. Furthermore, the patient rejected or was indifferent to advice from others. At the age of 61 years, he frequently coughed and experienced shortness of breath due to saliva and food; subsequently, he suffered from dysphagic pneumonia due to massive saliva and was finally readmitted to our hospital.

During the second hospitalization, the muscles of the patient's four limbs revealed more pronounced weakness and atrophy than during the first hospitalization. Generalized hyporeflexia was still present; however, bilateral Babinski reflexes were observed. A neuropsychological examination was conducted, when the patient improved after the pneumonia. The MMSE score was 21/30, whereas the MoCA score was 12/30 with disturbed attention, visuospatial cognition, and executive functions. The FAB score was 6/18 with disturbances of "similarities," "lexical fluency," "motor series," and "prehension behavior." The results of the neuropsychological tests revealed a deterioration of cognitive functions including mainly language and speech disturbances due to predominantly frontal and temporal lobe dysfunctions. His speech was apparently affected by non-fluent agrammatic primary progressive aphasia (naPPA) with word-finding difficulties and mistakes of words and characters. The changes in personality presented as adhesion, irritation, dependent tendencies, and self-centered behavior with childish manners. After the pneumonia improved, the patient was moved to another hospital, and his treatment continued. The patient was alert and could speak with the help of a speech cannula after a tracheotomy; however, he could also communicate independently with blinking. He needed frequent aspiration of saliva and oxygen inhalation to support his respiration. At the present age of 65 years, a lumbar puncture was performed, after we obtained the patient's informed consent.

The patient's mother had also shown muscular weakness and bilateral atrophy of the lower limbs at the age of 60 years, eventually also involving the upper limbs, which had resulted in her becoming bed-ridden. She was diagnosed with amyotrophic lateral sclerosis (ALS) and died from pneumonia at the age of 68 years; it was not confirmed whether she had been affected by dementia. The patient's father died from pancreatic cancer, whereas his elder sister suffered from gait disturbance of unknown etiology since her childhood and died from brain tumor at the age of 40 years. His younger brother died from malignant lymphoma at the age of 36 years. The patient did not have any children. During the first hospitalization, cerebral MRI showed bilateral frontal and temporal atrophy (**Figures 1A–C**). During the second

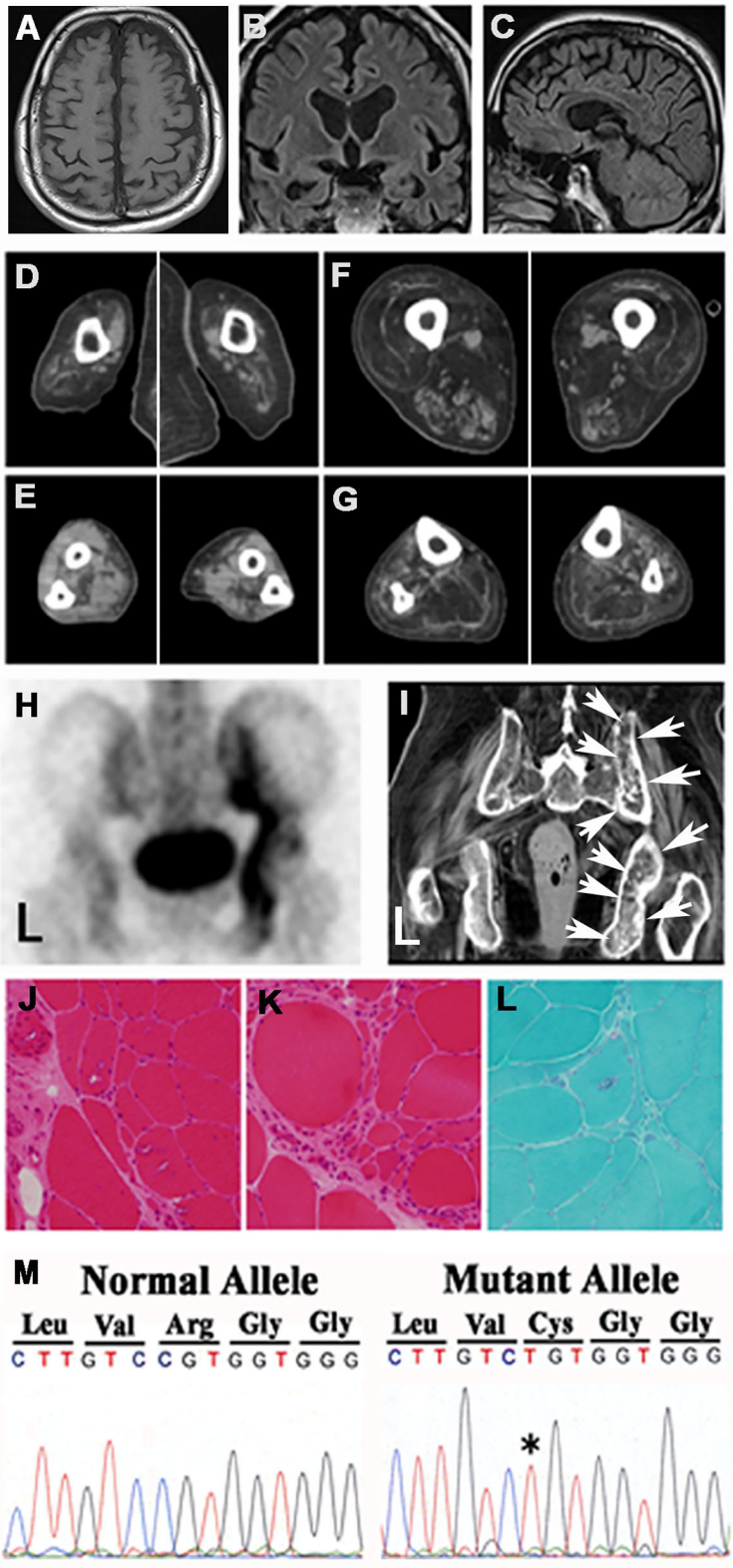

FIGURE 1 | Findings of images, pathological examinations, and DNA sequences. Cerebral MRIs of the **(A)** transverse view, **(B)** coronal view, and **(C)** sagittal view demonstrated frontal and temporal lobe atrophy. CT of muscles of the **(D)** upper arms, **(E)** forearms, **(F)** thighs, and **(G)** lower legs showed muscle atrophy in the four

(Continued)

FIGURE 1 | extremities. **(H)** ^{99m}Tc-HMDP bone scintigraphy of the pelvis revealed active osteoblastic accumulation in the right medial iliac bone. **(I)** Pelvic CT showed remodeling changes in the corresponding area designated with arrows **(H)**. Microscopic findings. Hematoxylin eosin staining showed multiple rimmed vacuoles in muscle cells **(J)** and numerous small fibers **(K)**. **(L)** Gomori trichrome staining demonstrated rimmed vacuoles and small angulated fibers. **(M)** Genomic DNA analysis revealed a missense mutation in the *VCP* gene that exchanged CGT (Arg) to T*GT (Cys).

TABLE 1 | Demographic characteristics of the patients with IBMPFD and neurodegenerative diseases and of non-dementia control subjects.

	IBMPFD	bvFTD	FTD-MND	PSP	CBS	AD	CTR
No.	1	7	5	7	7	24	18
Male	100	71.43	40.00	42.86	57.14	45.83	50.00
Age at onset (years old)	48	52 ± 3.24	60 ± 3.22	69 ± 1.93	68 ± 2.38	64 ± 1.58	–
Age at CSF analysis	#1: 52 #2: 65	55 ± 2.66	62 ± 2.82	71 ± 1.90	70 ± 2.53	69 ± 1.49	65 ± 2.34
MMSE (/30)	#1: 26 #2: 21	14 ± 3.58	18 ± 1.59	22 ± 2.28	16 ± 3.24	20 ± 1.08	29 ± 0.25
MoCA (/30)	#1: 18 #2:12	6 ± 2.76	13 ± 2.40	19.5 ±4.25	14 ± 4.87	16 ± 0.99	28.5 ± 0.36
FAB (/18)	#1: 8 #2: 6	8.5 ± 3.80	6.5 ± 1.31	7 ± 1.03	9 ± 2.65	9.5 ± 0.67	17 ± 0.28
NFL (pg/ml)	#1: 5,255.24 #2: 5,394.98	5,493.71 ± 814.18	9,371.82 ± 1,134.69	4,413.78 ± 741.49	4,217.29 ± 936.81	1,531.70 ± 167.56	452.93 ± 58.90
YKL-40 (ng/ml)	#1: 125.03 #2: 132.41	146.07 ± 25.87	154.39 ± 62.41	99.94 ± 17.62	84.59 ± 17.68	107.23 ± 10.26	60.53 ± 5.43
P-Tau (pg/ml)	#1: 31.79 #2: 34.72	41.75 ± 6.10	31.45 ± 6.87	36.60 ± 3.61	33.93 ± 7.08	76.42 ± 7.73	25.52 ± 2.53
T-Tau (pg/ml)	#1: 148.60 #2: 157.16	319.93 ± 50.07	213.99 ± 68.27	231.09 ± 63.39	90.61 ± 50.96	506.86 ± 71.60	143.56 ± 16.07

Clinical information and CSF data are described for this patient; the patients with neurodegenerative diseases (bvFTD, FTD-MND, PSP, CBS, and AD) and the CTR subjects. Patient #1: the first hospitalization, #2: the present hospitalization. The data represent median ± standard error (S.E.).

hospitalization, CT of the extremities exhibited severe bilateral muscle atrophy of the upper arms, forearms, thighs, and lower legs (**Figures 1D–G**). During the second hospitalization, ^{99m}Tc-HMDP bone scintigraphy showed active osteoblastic accumulation in the right medial iliac bone (**Figure 1H**), whereas pelvic CT revealed remodeling changes in the corresponding area indicated by arrows (**Figure 1I**). Hematoxylin and eosin staining of the muscle biopsy specimens demonstrated multiple rimmed vacuoles in muscle cells (**Figure 1J**) and numerous small fibers and round-shaped fibers (**Figure 1K**). Gomori trichrome staining showed rimmed vacuoles in muscle cells and small angulated fibers (**Figure 1L**), which were compatible with the pathological findings of IBMPFD during the first hospitalization. DNA analysis revealed a cytosine (C) to thymine (T) (C→T*) mutation, resulting in an amino acid exchange of arginine to cysteine (p.R155C) (**Figure 1M**) as previously described (2, 3, 9–14).

The neurological finding of this case revealed general muscle weakness and atrophy, especially, proximal muscles of lower extremities, progressive cognitive decline, speech disturbance, and character change. In muscle biopsy, rimmed vacuoles were pathologically confirmed and neurogenic muscle changes were also observed. ^{99m}Tc-HMDP bone scintigraphy of the patient was compatible with Paget's disease of bone (PDB).

METHODS AND RESULTS OF THE CSF ANALYSES

The patient was examined by lumbar puncture two times (during the present hospitalization and 13 years earlier). The CSF samples obtained from the patient by the two lumbar punctures were stored separately in 1.5-ml Eppendorf tubes. The CSF samples were strictly stored in a −80°C freezer and never opened nor freeze-thawed, until they were measured using enzyme-linked immunosorbent assay (ELISA) kits. Phosphorylated Tau (P-Tau), human total tau (T-Tau), neurofilament light chain (NFL), and YKL-40 were measured. The CSF samples were analyzed in this patient, patients with neurodegenerative diseases (bvFTD: $n = 7$, FTD-MND: $n = 5$, PSP: $n = 7$, CBS: $n = 7$, and AD: $n = 24$), and non-dementia control subjects (CTR: $n = 18$). The patients with IBMPFD, bv-FTD, FTD-MND, PSP, CBS, and AD were diagnosed in accordance with the global clinical criteria [IBMPFD (1, 2, 15); bv-FTD and FTD-MND (16–21); PSP (22, 23); CBS (24); and AD (25–27)] by experienced neurologists

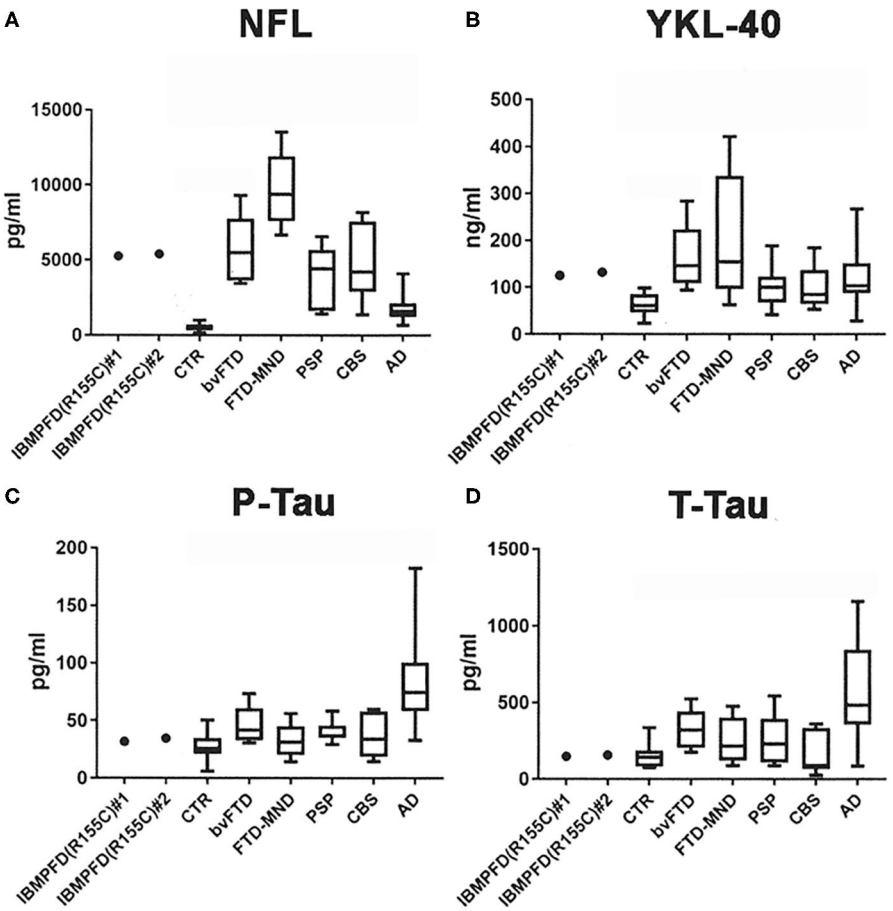

FIGURE 2 | CSF analyses of NFL, YKL-40, phosphorylated tau 181 (P-Tau), and total human tau (T-Tau). **(A)** Both CSF NFL levels in the patient (#1: the first hospitalization and #2: the present hospitalization) were higher than those in CTR and lower than those in FTD-MND; further, the CSF NFL levels in bvFTD, PSP, CBS, and AD were higher than those in CTR. **(B)** Both CSF YKL-40 levels in the patient (#1 and #2) were higher than those in CTR; moreover, CSF YKL-40 levels in FTD-MND, bvFTD, PSP, and AD were higher than those in CTR. **(C)** CSF P-Tau levels in AD were higher than those in CTR and in neurodegenerative diseases including the patient (#1 and #2). **(D)** CSF T-Tau levels in AD were higher than those in CTR and other neurodegenerative diseases including the patient (#1 and #2). Bars in each graph present mean data.

(M.I., T.K., H.K., M.F., K.M, K.N., Y.F., and Y.I.) at the Department of Neurology, Gunma University Hospital (**Table 1**). ^{99m}Tc-HMDP bone scintigraphy findings in IBMPFD patients were evaluated by senior radiologists (T.H. and Y.T.). P-Tau and T-Tau in CSF were analyzed with sandwich ELISA INNOTEST® PHOSPHO-TAU(181P) (Fujirebio Europe N.V., Gent, Belgium) (28, 29) and sandwich ELISA INNOTEST® T-Tau-Ag (Fujirebio Europe N.V., Gent, Belgium) (30), respectively. NFL and YKL-40 CSF levels were measured utilizing sandwich ELISA NF-light® (IBL International, Hamburg, Germany) (4–7) and MicroVue™ YKL-40 EIA kits (Quidel, San Diego, CA, USA) (7, 8), respectively.

The NFL CSF levels (pg/ml) in the patient were comparable at the two measurement points separated by 13 years (#1: the first puncture and #2: the second puncture). Both NFL CSF levels in the patient (#1: 5255.24 and #2: 5394.98) were higher than those in CTR individuals [452.93 ± 58.90; median ± standard error (S.E.)] and AD patients (1,531.70 ± 167.56), lower than those in FTD-MND patients (9,371.82 ± 1,134.69), and comparable to those in bvFTD (5,493.71 ± 814.18), PSP (4,413.78 ± 741.49), and CBS patients (4,217.29 ± 936.81; **Figure 2A**). The YKL-40 CSF levels (ng/ml) in the patient were comparable at the two times points (#1: the first time 125.03 and #2: the second time 132.41); furthermore, they were higher than those in CTR individuals (60.53 ± 5.43) and comparable to those in FTD-MND (154.39 ± 62.41), bvFTD (146.07 ± 25.87), PSP (99.94 ± 17.62), and AD (107.23± 10.26) (**Figure 2B**). The P-Tau CSF levels (pg/ml) in the patient (#1: 31.79 and #2: 34.72) were comparable to those in CTR individuals (25.52 ± 2.53), bvFTD (41.75 ± 6.10), FTD-MND (31.45 ± 6.87), PSP (36.60 ± 3.61), and CBS (33.93 ± 7.08), whereas the P-Tau levels (76.42 ± 7.73) in CSF of AD patients were higher than those in CTR individuals and patients with other neurodegenerative diseases (**Figure 2C**). The CSF levels of T-Tau (pg/ml) in the patient (#1: 148.60 and #2: 157.16) were comparable to those in CTR individuals (143.56 ± 16.07) and patients with other neurodegenerative diseases, whereas the

CSF levels of T-Tau in AD patients (506.86 ± 71.60) were higher than those in CTR individuals (143.56 ± 16.07) and patients with other neurodegenerative diseases (**Figure 2D**). The CSF levels of both P-Tau and T-Tau in the patient were comparable to those in bvFTD, FTD-MND, PSP, and CBS patients (**Figures 2C,D**). These data are presented in **Table 1**.

DISCUSSION

IBMPFD is clinically characterized by adult-onset muscle weakness and atrophy, early-onset PDB, and frontotemporal dementia (FTD) (1, 2, 15). *VCP* is identified as the most predominant causative gene among IBMPFD patients, and the R155C mutation has been reported including Japanese ethnic background (2, 3, 9–14). VCP-related IBMPFD represents a unique class D subtype of the neurodegenerative diseases named TDP-43 proteinopathies with numerous ubiquitin-positive neuronal intranuclear inclusions and dystrophic neurites (31–33). Recently, CSF NFL has been investigated as a diagnostic marker of axonal neurodegeneration, especially ALS and frontotemporal lobar degeneration (FTLD) including bvFTD, PSP, CBS, and AD (5–7). Furthermore, YKL-40 has been identified as a CSF biomarker of glial neuroinflammation in ALS, FTLD, PSP, CBS, and AD (6–8).

This is the first report of a Japanese IBMPFD patient demonstrating higher and comparable levels, over 13 years, of the CSF biomarkers NFL and YKL-40 in an IBMPFD patient with a *VCP* mutation than in CTR individuals. Up to date, there is no other report but this case at least within the Japanese Consortium for Amyotrophic Lateral Sclerosis Research (JaCALS). The symptoms of this patient were not compatible with the typical ALS phenotype; however, the patient showed neurogenic changes in the EMG examination (data not shown) and neurogenic pathological changes in muscle biopsy. This patient is clinically expected to have poor prognosis, because his respiratory function has gradually deteriorated due to progressive general muscle weakness and atrophy due to IBMPFD. The patient will still require frequent aspirations of saliva and oxygen inhalation to support his respiration.

VCP mutations presumably lead to a dominant negative loss or alteration of VCP function culminating in impaired degradation of TDP-43 (34). Whereas IBMPFD is a multisystem proteinopathy (35), mutant VCP proteins are reportedly targets of autophagic-lysosomal degeneration, mitochondrial dysfunction, and ubiquitin–proteasome system disorders (36). A limitation of this study is the fact that only one patient of IBMPFD with a *VCP* mutation was included, which impeded statistical analyses for the other neurological diseases and CTR groups. NFL and YKL-40 levels were not compared in blood samples among the patient, noncarriers, and asymptomatic carriers with a *VCP* mutation to prove the utility of blood biomarkers for IBMPFD.

Higher NFL and YKL-40 CSF levels in the IBMPFD patient with a *VCP* mutation may be related to both axonal neurodegeneration and glial neuroinflammation. The implicated multifaceted pathological mechanisms should be elucidated, which may allow the discovery of new therapeutic targets for the *VCP* gene and/or the VCP protein in IBMPFD.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by this study was approved by the ethics committee of the Gunma University Hospital (Masaki Ikeda) and the Jobu Hospital for Respiratory Diseases (Takeo Kuwabara). The patients/participants provided their written informed consent to participate in this study.

CONSENT FOR PUBLICATION

Written consent to publish the clinical information was obtained from the patient's family.

AUTHOR CONTRIBUTIONS

MI and TK collected the clinical data and interpreted the data, and MI wrote the manuscript. ET analyzed the genomic DNA from the patient's blood samples and CSF biomarkers from the patient's CSF. YF performed the pathological examinations and evaluated the results. TH and YT evaluated the neuroimaging information. HK, MF, KM, AS, KN, and TY discussed the clinical information in terms of neurological features. MI and YI performed the clinical data analysis and evaluated their specificity and neurological significance. All authors contributed to the article and approved the submitted version.

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REVIEW
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Epigenetics: Recent Advances and Its Role in the Treatment of Alzheimer's Disease

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Objective: This review summarizes recent findings on the epigenetics of Alzheimer's disease (AD) and provides therapeutic strategies for AD.

Methods: We searched the following keywords: "genetics," "epigenetics," "Alzheimer's disease," "DNA methylation," "DNA hydroxymethylation," "histone modifications," "non-coding RNAs," and "therapeutic strategies" in PubMed.

Results: In this review, we summarizes recent studies of epigenetics in AD, including DNA methylation/hydroxymethylation, histone modifications, and non-coding RNAs. There are no consistent results of global DNA methylation/hydroxymethylation in AD. Epigenetic genome-wide association studies show that many differentially methylated sites exist in AD. Several studies investigate the role of histone modifications in AD; for example, histone acetylation decreases, whereas H3 phosphorylation increases significantly in AD. In addition, non-coding RNAs, such as *microRNA-16* and BACE1-antisense transcript (*BACE1-AS*), are associated with the pathology of AD. These epigenetic changes provide us with novel insights into the pathogenesis of AD and may be potential therapeutic strategies for AD.

Conclusion: Epigenetics is associated with the pathogenesis of AD, including DNA methylation/hydroxymethylation, histone modifications, and non-coding RNAs, which provide potential therapeutic strategies for AD.

Keywords: Alzheimer's disease, epigenetic, DNA methylation, DNA hydroxymethylation, histone modifications, non-coding RNA (ncRNA)

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INTRODUCTION

Alzheimer's disease (AD) is a devastating neurological disease characterized by progressive cognitive impairments. As the most common form of dementia in the world, AD accounts for an estimated 60–80% of dementia cases worldwide (1). It is estimated that 50 million people are living with dementia worldwide currently, and the figure will increase to 152 million by 2050 (2). The common hypotheses of AD include amyloid cascade hypothesis, tau propagation, neuroimmune activation, mitochondrial cascade hypotheses, and infectious hypothesis (3).

AD can be classified into familial and sporadic AD based on family history. Three causative genes, including presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), and amyloid precursor protein (*APP*) are involved in the pathogenesis of familial AD in an autosomal-dominant trait (4, 5). *PSEN1* is the

most common genetic cause in familial AD, and the second involved gene is *PSEN2*. *PSEN1* and *PSEN2* encode the presenilins, which are catalytic subunits of BACE1. *APP* is the third involved gene that encodes the APP from which amyloid-β peptide is cleaved. These genetic mutations trigger the cascade of amyloid-β deposition, resulting in cognitive impairments in patients with AD (6, 7).

The genetics of sporadic AD are much more complex than that of familial AD. With the developments of genetic sequencing technology, particularly the Genome-Wide Association Study (GWAS), scientists have identified a number of loci containing susceptibility alleles in sporadic AD. One of the most important loci is the apolipoprotein E gene (*APOE*), and the three major isoforms are *APOE* ε2, ε3, and ε4 based on two-point mutations (rs429358 and rs7412). The *APOE* ε4 haplotype increases the likelihood of the onset of AD (8). Since 2009, tens of GWAS studies on AD have identified more than 20 novel loci including *ABCA7, BIN1, CD33, CLU, CR1, CD2AP, EPHA1, MS4A6A-MS4A4E, PICALM,* and *SORL1*, among others (9, 10).

Currently, much attention has been drawn to the nongenetic factors. Nongenetic factors are of paramount significance in the etiology of AD. Increasing evidence identifies multiple nongenetic factors for AD, most of which are related to lifestyle. A systematic review and meta-analysis based on the current evidence proposes 21 nongenetic factors for the prevention of AD, such as low level of education, hypertension, hyperhomocysteinemia, diabetes, obesity in late life, depression, and stress (11). The underlying mechanisms of how these nongenetic factors affect AD are not fully understood. The common hypotheses include oxidative stress, inflammation, brain reserve theory, the hypoperfusion hypothesis, and hypomethylation theory (12). In the 5xFAD mouse model, environmental enrichment, a combination of cognitive and sensory stimulation and social interaction, improves cognitive impairment via altering epigenetic markers (13).

Epigenetics may explain the roles of non-genetic factors involved in AD and help us to better understand the etiology of AD. Epigenetics was so named first by Conrad Waddington in the 1940's, and it is now defined as the study of molecules and mechanisms that perpetuate alternative gene activity states without changing the DNA sequence (14). Specifically, epigenetics includes DNA methylation/hydroxymethylation, histone modifications, and non-coding RNA regulation. These modifications play a crucial role in the gene readout, such as gene silencing, transcription, and post-transcriptional RNA processing. Therefore, epigenetics makes a significant impact on the disease (15, 16). Evidence shows that epigenetics is involved in the pathogenesis of AD (**Figure 1**). Here, we review recent findings on the epigenetics of AD and its potential therapeutic strategies.

DNA METHYLATION/ HYDROXYMETHYLATION IN AD

DNA methylation refers to methylation of the nucleobases in DNA by a set of proteins called DNA methyltransferases (DNMTs). Most of the methylated nucleobases are cytosines in CpGs and CpHs (H = A, T, C), forming 5-methylcytosine (5 mC). Five mC is mostly enriched in CpG dinucleotides (17). Although a lot of attention on DNA methylation focuses on CpGs, CpHs methylation is found to be associated with gene expression in neurons (18). In addition to 5 mC, methylation of another nucleobase is identified. In mouse prefrontal cortical neurons, deoxyadenosine methylation on N6 (m6dA) is correlated with gene expression and the formation of memory (19). DNMTs include DNA methylTransferase 1, DNA methylTransferase 3A, and DNA methylTransferase 3B (20). DNA hydroxymethylation is the hydroxymethylation of the cytosine to develop 5-hydroxymethylcytosine (5 hmC), and 5 hmC is converted from 5 mC by ten-eleven translocase (TET) isoforms (21). CpG and CpH are abbreviations for the cytosine and other nucleobases divided by a phosphate. The methyl/hydroxymethyl groups are located in the major groove of the DNA helix where many DNA-binding proteins make contact. Therefore, DNA methylation can lead to gene silencing, X-chromosome inactivation, and genomic imprinting. The exact functions of DNA hydroxymethylation remain unclear, and it may promote or repress gene expression by binding to regulatory regions of a gene (22, 23).

To date, a variety of studies have analyzed the role of DNA methylation in AD. One of the most studied genes of DNA methylation is the *APP* gene. CpG dinucleotides are enriched in the 5′ region of the *APP* gene, and alteration of its methylation levels can affect APP expression. Some studies identify hypomethylation of the *APP* gene in patients with AD compared with normal controls by analyzing postmortem brains or peripheral blood leucocytes *in vitro* (24–26), whereas, two other studies show no differences between AD and normal controls (27, 28). The reasons for the different results are not well-understood. Possible explanations include different test methods, different tissues examined, and relatively small sample sizes (29). Specifically, these studies examine the *APP* methylation levels in postmortem brain tissues or blood from a few AD patients and normal controls. Large-scale studies on methylation of the *APP* gene may address such different results in the future. Meanwhile, by analyzing genomic DNA, there are markedly different methylation levels of the *APP* gene in different human tissues (30). Therefore, the different tissues examined may also account for the different findings in the *APP* methylation levels. Furthermore, methylcytosines are reduced in the *APP* gene with age, which may be associated with Aβ deposition in AD (31). In addition to age, gender affects the methylation levels of the *APP* gene. Hypermethylation of the *APP* gene is identified in the female mouse cerebral cortex compared to male mice (32).

PSEN1 and *PSEN2* genetic methylation patterns do not differ significantly between AD samples and normal controls (27, 28). After adjusting for gender and *APOE*, there are no significant methylation levels of *PSEN1* and *PSEN2* between AD patients and normal controls (33). No difference in *PSEN1* methylation levels is observed in the AD mouse model, suggesting that Aβ production is not associated with *PSEN1* methylation (34). Nevertheless, reduced methylation of the *PSEN1* gene is identified in the human cerebral cortex (35, 36). In blood DNA, *PSEN1* methylation is significantly downregulated in AD patients compared to normal controls, which is associated with

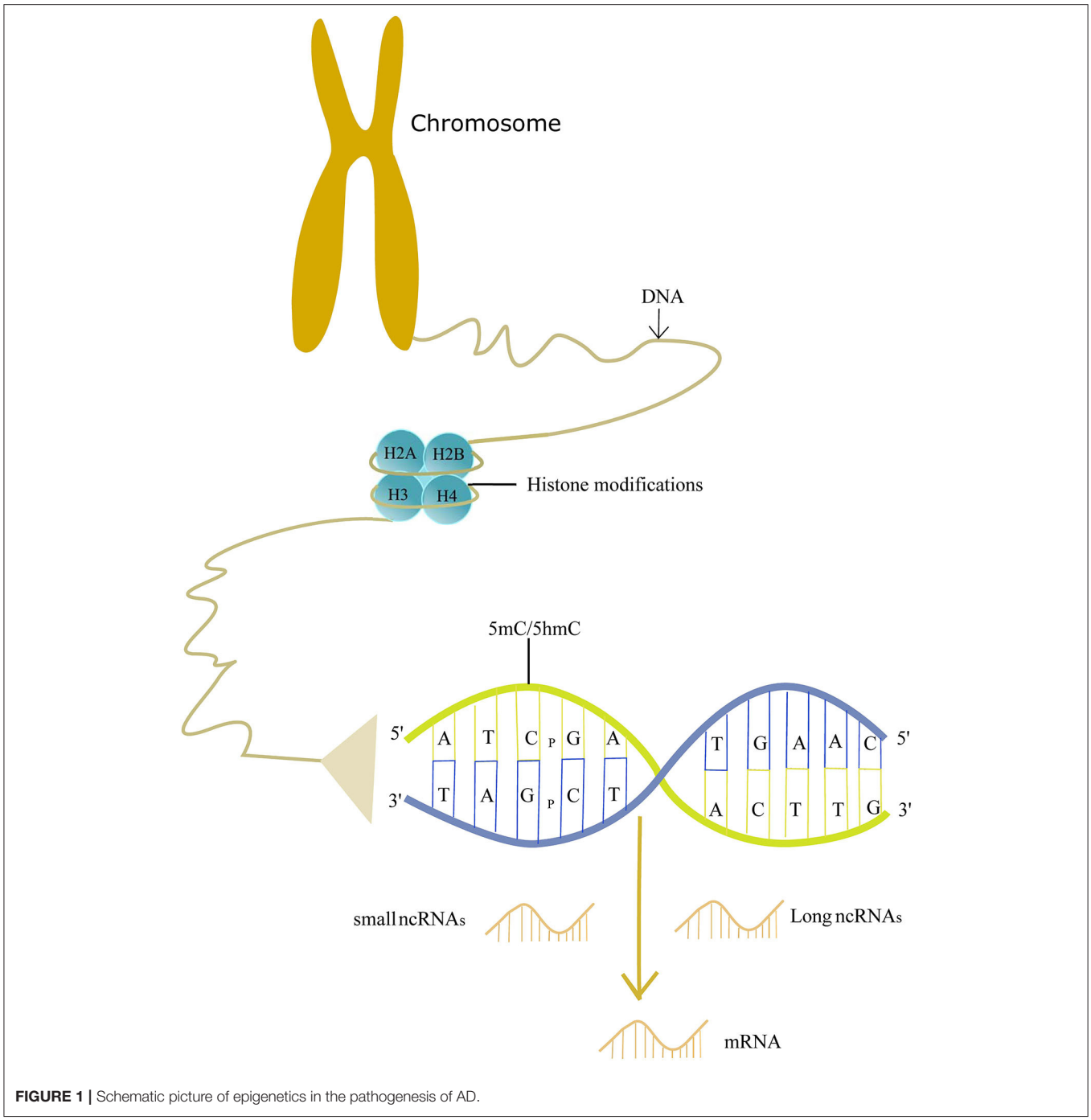

FIGURE 1 | Schematic picture of epigenetics in the pathogenesis of AD.

higher *PSEN1* expression during the progression of AD (37). The inconsistent findings may be associated with significant interindividual methylation variance of the *PSEN1* gene (38). For *APOE*, two studies show that there is no significant difference in DNA methylation levels in postmortem brain tissues between AD patients and normal controls (39, 40). However, Foraker et al. observe a significant decrease in DNA methylation levels of *APOE* by evaluating methylation profiles of AD postmortem brains *in vitro* (41). The reason for this inconsistency may result from the detection platform. The previous two studies mainly focus on *APOE* promoter regions lacking CpG islands based on bead-chip. In the subsequent study, CpG methylation of *APOE* is obtained by pyrosequencing, which is more reliable in reflecting *APOE* methylation levels (41). Another study demonstrates that non-neuronal cells mainly contribute to the low levels of *APOE* DNA methylation in AD patients (42).

Further, a lot of studies examine methylation sites of candidate susceptibility genes for AD (**Figure 2**). An et al. show that the

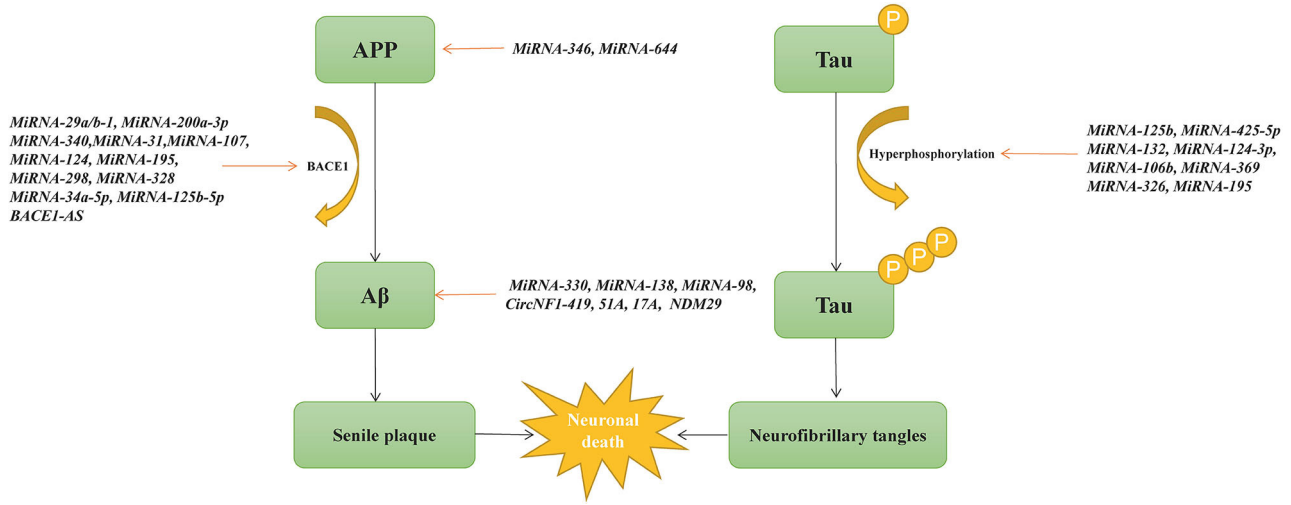

FIGURE 2 | Specific gene methylation in AD: candidate gene approach.

2′,5′-oligoadenylate (2-5A) synthetase gene is hypomethylated in AD cells *in vitro* (43). Sanchez-Mut et al. show that *TBXA2R, SORBS3*, and *SPTBN4* are hypermethylated in 12 distinct AD mouse brain regions, suggesting that the axon initial segment and cAMP response element-binding protein (CREB) activation pathway is involved in AD pathogenesis (44). Lei Yu et al. demonstrate that DNA methylation levels in *SORL1, ABCA7, HLA-DRB5, SLC24A4*, and *BIN1* are associated with greater odds for pathological AD by examining 28 reported AD loci discovered from recent GWAS reports on AD, which indicate that the change of DNA methylation of AD risk genes contribute to the etiology of AD (45). The hypermethylation of *BDNF* is repeatedly observed in AD patients' blood samples, which might be a diagnostic marker of AD (46, 47). Nevertheless, Carboni et al. find that *BDNF, SIRT1*, and *PSEN1* exhibit no different methylation patterns in AD compared with controls using peripheral blood samples *in vitro*. The inconsistency among the studies may result from methodological differences. Bisulphite pyrosequencing technology and methylation-specific primer real-time PCR cover different CpGs when evaluating CpG sites in *BDNF* (48).

Fabio Coppedè et al. show that there are no methylation differences in DNA repair genes between patients with AD and non-affected individuals, including *OGG1, PARP1, MRE11A, BRCA1, MLH1*, and *MGMT* (49). Ma et al. find that *UQCRC1* is highly methylated in patients with AD by studying peripheral blood samples of AD, suggesting that inflammation and oxidative stress may contribute to AD (50). The DNA methylation level of *PLD3* is increased and correlated with hippocampal Aβ in AD hippocampus compared to controls (51). A lower DNA methylation level at *TREM2* is observed in AD patients and associated with TREM2 mRNA expression, which may be a biomarker for AD (52). *CRTC1* is hypomethylated in the AD hippocampus and associated with p-tau deposition (53). Hypomethylation of CpGs in *BIN1* is identified in participants with AD and confers risk to AD by studying peripheral blood, indicating it may be a biomarker for AD (54). The methylation level of *BIN1* is associated with neuritic plaque pathology in the peripheral blood (55). Furthermore, the methylation levels of *OPRM1* and *OPRL1* are significantly increased in AD compared to controls, suggesting that opioid receptor genes may be potential biomarkers for diagnosing AD (56). The DNA methylation level of *PICALM* is decreased and linked to cognitive decline in AD (57). Elevated *ANK1* DNA methylation exists in the entorhinal cortex of AD and is associated with AD pathology (58). Similarly, another study also identifies that hypermethylated *ANK1* is observed in the entorhinal cortex, superior temporal gyrus, and prefrontal cortex (39). Methylation of *ABCA2* is negatively associated with AD risk and may be a therapeutic target of AD (59). Furthermore, other studies identify many genes without methylation changes between AD and normal controls, such as *SORL1, SIRT1, SST, SSTR4, HSPA8, HSPA9, SIRT3*, and *ABCA7* (40, 60–63) (**Table 1**).

Recently, there were several genome-wide methylation analyses of AD. By examining genome-wide enhancer methylation levels in the prefrontal cortex of AD patients, Li et al. identify 1,224 enhancer regions methylated differentially and most of their methylation levels are decreased in AD neurons, which are associated with Aβ, tau, and cognitive impairment (65). Humphries et al. show that 1,106 of 5,147 CpG sites differ between LOAD patients and controls, 87.3% of which are hypomethylated and related to the myelination network in LOAD (66). Moreover, Watson et al. identify 479 differentially methylated regions in patients with AD compared to normal controls by performing a genome-wide screen of DNA methylation in the temporal gyrus of AD, and 475 of these regions are involved in neuron function and development (67). De Jager et al. show that 11 of 415,848 interrogated CpGs are significantly associated with the AD pathological burden by studying AD autopsied brains, including CpGs in *ABCA7*

TABLE 1 | Specific gene methylation and hydroxymethylation in Alzheimer's disease: candidate gene approach.

Gene	Function	Tissue type	Main finding	References
APP	Amyloid precursor protein	Human postmortem cerebellum, parietal lobe and temporal lobe	Hypomethylation	(24)
APP	Amyloid precursor protein	Human postmortem temporal lobe	Hypomethylation	(25)
APP	Amyloid precursor protein	Human peripheral blood samples	Hypomethylation	(26)
APP/PSEN1/PSEN2	Amyloid precursor protein/component of γ-secretase	Human postmortem frontal cortex and hippocampus	No differences	(27)
APP/PSEN1/PSEN2	Amyloid precursor protein/component of γ-secretase	Human postmortem frontal cortex, parietal cortex, temporal cortex, and cerebellum	No differences	(28)
PSEN1/PSEN2	Component of γ-secretase	Human peripheral blood	No differences	(33)
PSEN1	Component of γ-secretase	Human postmortem cerebral cortex	Hypomethylation	(35)
PSEN1	Component of γ-secretase	Human postmortem cerebral cortex	Hypomethylation	(36)
PSEN1	Component of γ-secretase	Human peripheral blood	Hypomethylation	(37)
APOE	Risk gene for AD	Human postmortem entorhinal cortex, cerebellum, superior temporal gyrus and prefrontal cortex	No differences	(39)
APOE	Risk gene for AD	Human postmortem brain tissues (entorhinal and auditory cortices and hippocampus)	No differences	(40)
APOE	Risk gene for AD	Human postmortem cerebellum, hippocampus	Hypomethylation	(41)
2′,5′-oligoadenylate (2-5A) synthetase	An enzyme induced by interferon (IFN)	Human skin fibroblasts	Hypomethylation	(43)
SORL1, ABCA7, HLA-DRB5, SLC24A4, and *BIN1*	Risk gene for AD	Human dorsolateral prefrontal cortex tissue	Their methylation was associated with AD pathologies	(45)
BDNF	Nerve growth factor	Human peripheral blood samples	Hypermethylation	(46)
BDNF	Nerve growth factor	Human peripheral blood samples	Hypermethylation	(47)
BDNF, SIRT1, and *PSEN1*	Nerve growth factor/Sirtuin 1/ Component of γ-secretase	Human peripheral blood samples	No difference	(48)
OGG1, PARP1, MRE11A, BRCA1, MLH1, and *MGMT*	DNA repair	Human peripheral blood samples	No differences	(49)
UQCRC1	A subunit of the respiratory chain protein	Human peripheral blood samples	Hypermethylation	(50)
PLD3	Risk gene for AD	Human hippocampal samples	Hypermethylation	(51)
TREM2	Risk gene for AD	Human peripheral blood samples	Hypomethylation	(52)
CRTC1	CREB regulated transcription coactivator 1	Human postmortem hippocampus	Hypomethylation	(53)
BIN1	Risk gene for AD	Human peripheral blood samples	Hypomethylation	(54)
OPRM1 and *OPRL1*	Opioid receptor genes	Human peripheral blood samples	Hypermethylation	(56)
PICALM	Risk gene for AD	Human peripheral blood samples	Hypomethylation	(57)
ANK1	Encoding for ankyrin-1	Human postmortem entorhinal cortex	Hypermethylation	(58)
ABCA2	Risk gene for AD	Human peripheral blood samples	Methylation of *ABCA2* was negatively associated with AD risk	(59)
SORL1 and *SIRT1*	Risk gene for LOAD	Human postmortem brain tissues (entorhinal and auditory cortices and hippocampus) and peripheral blood leukocytes	No difference	(40)
SST and *SSTR4*	Somatostatin and its receptor	Human postmortem brain tissue (middle temporal and superior frontal gyrus)	No difference	(60)
HSPA8 and *HSPA9*	Chaperone	Human postmortem brain tissue (entorhinal and auditory cortices and hippocampus) and peripheral blood samples	No difference	(61)
PSEN1,BACE1,MTHFR, DNMT1, DNMT3A, and *DNMT3B*	*PSEN1* and *BACE1*: Aβ production *MTHFR*: one-carbon metabolism *DNMT1, DNMT3A,* and *DNMT3B*:DNA methylation	Human peripheral blood samples	No difference	(62)

(Continued)

TABLE 1 | Continued

Gene	Function	Tissue type	Main finding	References
ABCA7	Risk gene for AD	Human peripheral blood samples	No differences	(63)
TREM2	Risk gene for AD	Human postmortem hippocampus	Hyperhydroxymethylation	(64)
TBXA2R/SORBS3/SPTBN4	Family of G protein-coupled receptors/involved in synapsis/member of the axon initial segment	C57BL/6J mice and human postmortem frontal cortex	Hypermethylation	(44)
PSEN1	Component of γ-secretase	TgCRND8 mice brains and blood	No differences	(34)

BDNF, brain derived neurotrophic factor; SIRT1, Sirtuin 1; OGG1, 8-Oxoguanine DNA Glycosylase; PARP1, Poly-ADP-ribose polymerase-1; MRE11A, Meiotic Recombination 11 Homolog A; BRCA1, breast cancer 1; MLH1, MutL homolog 1; MGMT, O6-methylguanine-DNA methyltransferase; UQCRC1, Ubiquinol-Cytochrome C Reductase Core Protein 1; PLD3, phospholipase D family member 3; TREM2, Triggering receptor expressed on myeloid cells 2; CRTC1, CREB Regulated Transcription Coactivator 1; OPRM1, Opioid Receptor Mu 1; OPRL1, Opioid Related Nociceptin Receptor 1; PICALM, Phosphatidylinositol Binding Clathrin Assembly Protein; ANK1, Ankyrin 1; ABCA2, ATP Binding Cassette Subfamily A Member 2; SST, Somatostatin; SSTR4, Somatostatin Receptor 4; HSPA8, Heat Shock Protein Family A (Hsp70) Member 8; HSPA9, Heat Shock Protein Family A (Hsp70) Member 9; MTHFR, Methylenetetrahydrofolate Reductase; DNMT1, DNA Methyltransferase 1; DNMT3A, DNA Methyltransferase 3 Alpha; DNMT3B, DNA Methyltransferase 3 Beta.

and *BIN1* regions (68). Moreover, an epigenome-wide study identifies that DNA methylation of *OXT* is associated with the risk of AD, indicating it may be a novel promising biomarker or therapeutic target in AD (69). In another epigenome-wide DNA methylation study, among 17,895 differentially methylated CpG sites, there are 11,822 hypermethylated CpGs and 6,073 hypomethylated CpGs in the superior temporal gyrus of AD patients (70). When examining 420,852 DNA methylation sites from four brain regions in late-onset AD and neurotypical controls, 858 sites show differential methylation patterns, indicating that DNA methylation may contribute to AD (71). Altuna et al. profile genome-wide DNA methylation levels in the hippocampus of AD patients in which 118 AD-related differentially methylated positions are found, and these positions are linked to phosphorylated tau burden (72). The first integrated base-resolution genome-wide study finds 39 CpG site-specific and 27 AD region-specific epigenetic changes in AD, providing reliable epigenetic signatures for the diagnosis and treatment of AD (73) (**Table 2**).

Global DNA methylation/hydroxymethylation is the percentage of methylcytosine/hydroxymethylcytosine of total cytosine. The percentage of methylation of CCGG sites exhibits no significant difference by investigating DNA methylation in the human brain (78). Moreover, no significant global 5mC and 5hmC changes are found in the entorhinal cortex of AD patients (79). Some studies find that the levels of global 5 mC and 5 hmC is decreased in patients with AD (80–82). However, in the postmortem AD cortex, global levels of 5 mC and 5 hmC are significantly increased in AD samples and correlated with biomarkers of AD (83). Global increased 5 mC levels are identified in the postmortem frontal cortex from AD patients (84). By analyzing DNA isolated from peripheral blood, global DNA methylation levels are upregulated in AD patients and correlated with cognitive impairments (85). The reasons for the difference are far from being understood. One possible reason is that they investigated different brain regions. Nevertheless, some findings are contradictory even for the same test method and brain region. The differences in test method procedures and sample size may account for the different findings. In addition to studies in postmortem brain regions, global DNA methylation/hydroxymethylation was also investigated in the AD mouse model. The 5xFAD mouse model is composed of two *APP* and two *PSEN1* mutations. Global high levels of 5-mC and a reduction of 5-hmC were observed in the 5xFAD mouse model, which are related to cognition and paralleled with Aβ deposition (86). In the 3xTg-AD mouse model, global methylation and hydroxymethylation levels are reduced (87). In the SAMP8 mouse model, global DNA methylation levels are decreased while 5-hmC levels are upregulated (88).

Compared to DNA methylation, relatively few studies analyze the role of DNA hydroxymethylation in AD. As we mentioned previously, 5 hmC is the specific product of DNA hydroxymethylation. Five hmC is highly concentrated in the central nervous system and plays an important role in neurodevelopment and neurological function (89, 90). The triggering receptor expressed on myeloid cells (TREM2) is a receptor in brain microglia and contributes to the pathogenesis of AD (91). When examining DNA hydroxymethylation levels in *TREM2*, Celarain et al. find that the 5 hmC levels were increased in AD patients and involved in TREM2 mRNA expression (64) (**Table 1**). Shu et al. demonstrate that the 5 hmC levels are decreased in the hippocampus in a mouse model of AD, whereas another study finds that the 5 hmC levels are significantly increased in it in AD patients. Different tissue types may explain the contradictory findings (92, 93). The genome-wide distribution of 5 hmC finds that 517 differentially hydroxymethylated regions (DhMRs) are significantly associated with neuritic plaques, whereas 60 DhMRs are associated with neurofibrillary tangles (74). Moreover, there are 325 genes containing differentially hydroxymethylated loci in AD from genome-wide analyses of 5 hmC in the prefrontal cortex of postmortem AD patients, involving neuronal projection development and neurogenesis (75). By studying genome-wide profiles of 5 mC and 5 hmC profiles in frontal cortex tissues from Chinese AD patients, two significant transcription factor-binding motifs, hypoxia-inducible factor 2α, and hypoxia-inducible factor 1α are enriched in the differentially hydroxymethylated regions (76). As mentioned previously, DNA hypermethylation in *ANK1*

TABLE 2 | Specific gene methylation and hydroxymethylation in AD: Genome-wide approach.

Design and cases	Tissue type	Methylation sites	Main finding	References
101 individuals with no/mild, moderate and severe AD pathology (Braak stage: 1–2 $n = 38$ individuals, 3–4 $n = 32$, and 5–6 $n = 31$ individuals, respectively)	Human postmortem prefrontal cortex	1.2 million CpG and CpH sites in enhancers	1,224 differentially methylated enhancer regions; most of which are hypomethylated at CpH sites in AD neurons	(65)
AD cases ($N = 10$) compared to normal controls ($N = 10$) and disease controls (DLB $N = 10$)	Human postmortem temporal pole	5,147 CpG sites on 465 genes	1,106 of the 5,147 CpG sites differed between LOAD patients and controls, and 87.3% of them was hypomethylated in LOAD	(66)
AD patients ($N = 34$) and non-AD subjects ($N = 34$)	Human postmortem superior temporal gyrus	461,272 autosomal CpGs	479 differentially methylated regions in AD patients, and 475 of these regions are involved in neuron function, metabolism and development	(67)
Investigating AD methylation state with the burden of AD pathology prospectively (N=708)	Human postmortem dorsolateral prefrontal cortex	415,848 interrogated CpGs	11 of 415,848 interrogated CpGs are significantly associated with AD pathological burden, including CpGs in the *ABCA7* and *BIN1* regions	(68)
Comparison of AD patients (45) with age-matched controls ($N = 35$) and converters to AD dementia (N=54) and non-converters ($N = 42$)	Human postmortem middle temporal gyrus and peripheral blood samples	Epigenome-wide patterns of DNA 5 mC and 5 hmC	DNA methylation of *OXT* was associated with AD risk in the elderly	(69)
34 patients with late-onset AD and 34 controls without dementia	Human postmortem superior temporal gyrus	17,895 differentially methylated CpG sites	There were 11,822 hypermethylated CpGs and 6,073 hypomethylated CpGs	(70)
Late-onset AD patients ($N = 24$) and controls ($N = 49$)	Human postmortem hippocampus, entorhinal cortex, dorsolateral prefrontal cortex and cerebellum	420,852 DNA methylation sites	858 sites showed differential methylation patterns	(71)
26 AD patients and 12 control subjects	Human postmortem hippocampal samples	5-methylcytosine	118 AD-related differentially methylated positions were identified	(72)
371 AD patients and 163 control subjects	Normal and AD patient derived iPSCs, neural progenitor cells, and cortical neuronal cells	5-methyl-cytosine (5mC), 5-hydroxymethyl-cytosine (5 hmC), and 5-formyl/carboxy-cytosine (5fC/caC)	39 CpG site-specific and 27 AD region-specific epigenetic changes	(73)
Identifying AD DhMRs associated with AD pathology ($N = 30$)	Human postmortem dorsolateral prefrontal cortex tissue	5-hydroxymethylcytosine (5 hmC) at specific genomic loci	517 DhMRs significantly associated with neuritic plaques while 60 DhMRs associated with neurofibrillary tangles	(74)
Comparison of AD ($N = 6$) with control cases ($N = 5$)	Human postmortem frontal cortex	5-methylcytosine and 5-hydroxymethylcytosine (5 hmC)	There were 325 genes containing differentially hydroxymethylated loci in AD	(75)
Late-onset AD (LOAD) ($n = 5$) and neurologically normal controls ($n = 5$)	Human postmortem frontal cortex tissues	16,165 DhMRs annotated to 8,149 genes	HIF2 α and HIF1α was enriched in the DhMRs	(76)
96 individuals	Human postmortem cortex tissues	5-methylcytosine and 5-hydroxymethylcytosine (5 hmC)	Hypohydroxymethylation in ANK1 was found in entorhinal cortex of AD patients	(77)

DLB, Dementia with Lewy bodies; ATP, binding cassette subfamily A member 7; BIN1, box-dependent-interacting protein 1; OXT, Oxytocin; DhMRs: differentially hydroxymethylated regions; HIF2 α, hypoxia-inducible factor 2α; HIF1α, hypoxia-inducible factor 1α; ANK1, Ankyrin 1.

is identified in AD (58). Hypohydroxymethylation in *ANK1* is also found in the entorhinal cortex of AD patients in an epigenome-wide association study, further highlighting the significant role of *ANK1* in the development of AD (77) (**Table 2**).

It is increasingly becoming accepted that mitochondria play a significant role in the pathogenesis of AD (94). Blanch et al. show that mitochondrial 5 mC levels increase, whereas no significant differences in 5 hmC levels are identified in AD (95). However, another study finds that mitochondrial 5 hmC is significantly increased in the superior temporal gyrus of AD subjects (93). The different test methods among these studies may account for the different findings of mitochondrial 5 hmC in brain tissue. In APP/PS1 transgenic mice, the displacement loop mitochondrial methylation level is reduced while *12 S rRNA* gene mitochondrial methylation is increased, indicating that mitochondrial DNA methylation may play a role in the AD development (96).

HISTONE MODIFICATIONS IN AD

Histone is a kind of octamer consisting of pairs of H2A, H2B, H3, and H4, which form the nucleosome with DNA. Histone can be modified at the N-terminal tails, and the modifications can affect the three-dimensional structure of the chromatin, leading to the changes in the transcription of genes. The common histone modifications include acetylation, methylation, and ubiquitination. The modifications are controlled by a specific set of enzymes, such as acetyltransferases and deacetylases (97).

To date, there have been several studies of histone modifications in AD. A genome-wide study of H3K27 acetylation in AD observed 4,162 differential acetylomic variation peaks between AD patients and normal controls, which were associated with Aβ and tau pathology (98). Zhang et al. show that histone acetylation is significantly decreased in the temporal lobe of patients with AD compared with that of controls, which is consistent with the previous finding in an APP/PS1 mouse model of AD, highlighting that histone acetylation is involved in AD (99, 100). Narayan et al. demonstrate that acetyl histone H3 and H4 levels are significantly increased in postmortem AD brain tissue compared with normal controls by investigating global acetyl histone levels (101). Another study shows a significant increase of monocytic H4K12 acetylation in transgenic AD mouse models and MCI patients (102). Global histone H3 acetylation levels exhibit a significant increase in the frontal cortex in end-stage AD patients, supporting that histone acetylation plays an important role in AD (103). These studies highlight that histone acetylation levels are increased on a global scale in the AD brain. Anderson et al. observe notable decreases in methylation of H2B and H4 residues, whereas, the ubiquitination of H2B residue increases. These post-translational histone modifications are related to AD pathology and of great significance in the development of AD (104). With regard to the regulators of histone modifications, in APP/PS1 mice, age is associated with the global levels of histone modifications, suggesting that age is one of the main risk factors in the histone modifications of AD (105). An epigenome-wide association study demonstrates that tau protein affects histone acetylation changes and an altered chromatin structure in AD prefrontal cortices (106) (**Table 3**).

NON-CODING RNAs IN AD

Non-coding RNAs (ncRNAs) are defined as RNA molecules that are not translated into a protein. Less than 2% of the human genome encodes proteins, and the rest produces thousands of ncRNAs, including small ncRNAs (smaller than 200 nucleotides in length), such as microRNAs, small interfering RNAs, piwi-interacting RNAs, and a variety of long ncRNAs (longer than 200 nucleotides) (107).

One of the best characterized small ncRNAs in AD is microRNA. Many microRNAs are surrounded by the CpG island and located within exons, intergenic regions, or introns of genes (108). MicroRNAs can enhance or repress messenger RNA transcription by binding to the 3′-untranslated region or the promoter of the target gene (109). In sporadic AD, microRNAs play an important role in Aβ production and neurofibrillary tangle formation (**Figure 3**). Two studies demonstrate that *microRNA-16* inhibits the expression of APP both *in vitro* and *in vivo*, which may be a therapeutic target of AD (110, 111). As mentioned previously, BACE1 is one of the APP-cleaving enzymes in the production of Aβ. By investigating changes in 328 *microRNA* expression profiles, Hébert et al. find that *microRNA* cluster *microRNA-29a*/*b-1* suppressed endogenous BACE1 expression, and it is significantly decreased in sporadic AD patients (112). *MicroRNA-200a-3p* also reduces the expression of BACE1 and is confirmed to be decreased in the hippocampus of APP/PS1 and SAMP8 mice as well as in blood plasma from AD patients (113). *MicroRNA-340* is decreased in the hippocampus of an AD mouse model and associated with the overproduction of Aβ by targeting BACE1 (114). *MicroRNA-31* reduces the mRNA levels of BACE1 and improves memory deficits in AD triple-transgenic (3xTg-AD) female mice (115). Moreover, *microRNA-107*, *microRNA-124*, *microRNA-195*, *microRNA-298*, and *microRNA-328* are also associated with the expression of BACE1 (116–119). Another recent study shows that *microRNA-298* represses the expression of BACE1, APP, Aβ40, and Aβ42 in the cell model, suggesting that *microRNA-298* may be a therapeutic target for AD (120). Meanwhile, the addition of *microRNA-34a-5p* and *microRNA-125b-5p* reduces Aβ by targeting BACE1 (121). Abnormally phosphorylated tau protein is another key pathological hallmark of AD. Li et al. find that *microRNA−219-5p* is significantly upregulated in brain tissues of AD patients, contributing to tau phosphorylation and AD progression (122). *MicroRNA-125b* promotes the phosphorylation of tau by activating cyclin-dependent kinase 5 (CDK5) and p35/25, and *microRNA-125b* is increased in AD patients (123). Similarly, overexpression of *microRNA-125b* results in tau hyperphosphorylation by targeting phosphatases DUSP6 and PPP1CA (124). In HEK293/tau cells, *microRNA-425-5p* overexpression promotes tau phosphorylation through targeting HSPB8 in AD (125). *MicroRNA-132*, the most significantly downregulated microRNA in AD, is associated with lower levels of tau phosphorylation by regulating EP300,

TABLE 3 | Histone modifications in AD.

Histone modifications	Tissue type	Main findings	References
H3K27 acetylation	Human postmortem entorhinal cortex samples	4,162 differential acetylomic variation peaks between AD and normal controls	(98)
Histone acetylation	Human postmortem temporal lobe	Decreased significantly in AD	(99)
H3 and H4 acetylation	Human postmortem temporal gyrus	Increased significantly in AD	(101)
H3 acetylation	Human postmortem frontal cortex	Increased substantially in AD	(103)
H4 acetylation	Human postmortem frontal cortex	Decreased significantly in AD	(104)
H2B ubiquitination	Human postmortem frontal cortex	Increased significantly in AD	(104)
Histone acetylation	Hippocampus of AD APP/PS1 mouse	Decreased significantly in AD	(100)
H4K12 acetylation	Transgenic AD mouse models and MCI/AD patients monocytes	Increased significantly in AD	(102)

H3K27, histone H3 at lysine 27; H2B, histone 2B; H3, histone H3; H4, histone H4; H4K12: histone H4 at lysine 12; MCI, mild cognitive impairment.

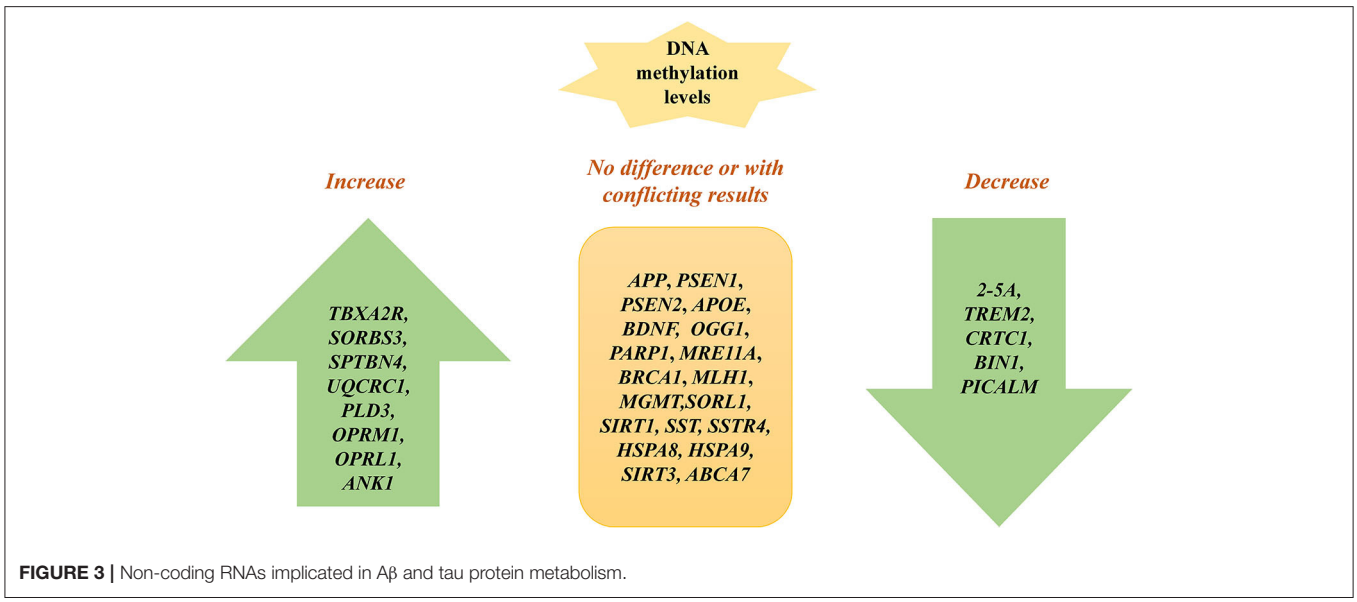

FIGURE 3 | Non-coding RNAs implicated in Aβ and tau protein metabolism.

GSK3b, Rbfox1, proteases Calpain2, and caspases 3/7 (126). *MicroRNA-124-3p* inhibits abnormal tau hyperphosphorylation through targeting the Caveolin-1-PI3K/Akt/GSK3β pathway in AD (127). *MicroRNA-106b* reduces tau phosphorylation induced by Aβ42 by involving the expression of Fyn (128). In 3xTg-AD mice, knocking out *microRNA-369* is associated with tau hyperphosphorylation via regulating Fyn and SRPK2 signaling pathways (129). *MicroRNA-326* decreases tau hyperphosphorylation and improves cognitive functions of AD through the JNK signaling pathway (130). Elevating the levels of *microRNA-195* diminishes tau hyperphosphorylation and Aβ burden in ApoE4$^{+/+}$ mice (131).

Furthermore, some altered microRNAs are directly related to APP or Aβ processing in AD. *MicroRNA-346* upregulates APP translation and Aβ production by binding to *APP* 5′UTR, and its levels change in late-stage AD patients (132). *MicroRNA-644* downregulate the formation of Aβ via targeting *APP* 3'UTR (133). Overexpression of *microRNA-330* reduces Aβ production and alleviates oxidative stress through the MAPK signaling pathway (134). In APP/PS1 mice, *microRNA-138* enhances Aβ production and improves cognitive impairment by decreasing the expression of sirtuin 1 protein (135). *MicroRNA-98* improves oxidative stress and downregulates Aβ production by activating the Notch signaling pathway (136). In addition to microRNAs, cortical circular RNAs (circRNAs) are associated with AD diagnosis, clinical dementia severity, and neuropathological severity, suggesting the potentially significant role of circRNA in the pathogenesis of AD (137). CircRNA *KIAA1586* is significantly enriched in AD-associated biological processes and may be a novel risk factor in the pathogenesis of AD (138). CircRNA *circ_0000950* promotes neuron apoptosis, inhibits the production of neurite outgrowth, and increases the levels of inflammatory cytokine levels via sponging microRNA-103 in AD (139). *CircNF1-419* upregulates autophagy and reduces Aβ and tau expression by binding the proteins Dynamin-1 and adaptor protein 2 B1 (140). *Circ-AXL*, *circ-GPHN*, and *circ-PCCA* differ significantly between AD patients and normal controls by studying the circRNA expression profile in cerebrospinal fluid,

which may be potential biomarkers in AD (141). In addition, 147 circRNAs are differentially expressed in different AD brain regions, most of which are found in the parahippocampal gyrus, supporting that circRNAs in the parahippocampal gyrus may be biomarkers in AD (142).

In the central nervous system, long ncRNAs (lncRNAs) are prevalent and play a critical role in the pathogenesis of AD (143). A conserved lncRNA called BACE1-antisense transcript (*BACE1-AS*) increases BACE1 mRNA stability and generates additional Aβ in AD patients as well as in APP transgenic mice, and knockdown of *BACE1-AS* improves memory (144, 145). The BACE1-AS level differs significantly between pre-AD and healthy controls as well as full-AD and healthy controls, indicating that BACE1-AS may be a potential biomarker of AD (146). LncRNAs, including *51A*, *17A*, and *NDM29*, increase the formation of Aβ and contribute to the pathogenesis of AD (147). LncRNA *SOX21-AS1* is upregulated in the AD mouse model, and its inhibition reduces neuronal oxidative stress and suppresses neuronal apoptosis via the Wnt signaling pathway (148). LncRNA *BC200* levels are enhanced significantly in AD brains and involved in dendritic loss by regulating local protein synthesis (149). Brain-derived neurotrophic factor antisense RNA(*BDNF-AS*) is an lncRNA that represses BDNF expression. The inhibition of *BDNF-AS* results in neuronal growth and differentiation, which may be the novel pharmacological target in AD (150). LncRNA *Sox2OT* represses *Sox2* gene expression, involving neurogenesis and neuronal differentiation (151). In APP/PS1 mice, lncRNA *EBF3-AS* is upregulated in the hippocampus and related to neuron apoptosis by regulating EBF3 expression (152). LncRNA *NAT-Rad18* increases the likelihood of apoptosis under DNA damage-related stress via targeting *RAD18* (153). LncRNA *TUG1* promotes neuronal apoptosis in the hippocampus by increasing microRNA-15a levels and suppressing ROCK1 expression (154). In the SAMP8 mouse model, lifestyle, including diet, exercise, and environmental enrichment, results in epigenetic changes (20). In addition, 3112 differentially expressed lncRNAs are identified in the SAMP8 mouse model, most of which are intergenic and exon sense-overlapping (155). In the human AD brain, 16 age-associated and 13 gender-associated lncRNAs are identified; among them, lncRNAs *SNHG19, LINC00672*, *RNF144A-AS1*, *LY86-AS1*, and *LINC00639* are associated with the pathology of AD (156). Nuclear paraspeckle assembly transcript 1 (*NEAT1*), a lncRNA that is widely expressed in cells, is of great importance in various biological and pathological processes by mediating target genes' expression (157). *NEAT1* is involved in Aβ clearance by regulating the expression of endocytosis-related genes in AD (158). Additionally, in an APP/PS1 transgenic mouse model, *NEAT1* is increased and promotes the pathogenesis of AD via upregulating PTEN-induced putative kinase 1 (PINK1)'s ubiquitination and degradation, which provided a potential therapeutic strategy in AD (159) (**Table 4**).

The mechanisms underlying epigenetic regulation of non-coding RNAs in AD are complex. As we mention, circRNAs mediate the effect of microRNAs (139). MicroRNAs are regulated by chromatin modifications and DNA methylation, which are implicated in AD by targeting messenger RNA (mRNA) expression (182). Dysregulated circRNAs are associated with the increased number of downstream target mRNAs in the Tg2576 AD mouse model, indicating that circRNA-microRNA-mRNA may play a significant role in the pathogenesis of AD (183). Emerging evidence shows that lncRNAs are involved in multiple epigenetic processes, such as DNA methylation, via regulating the interactions of target genes with chromatin-remodeling enzymes. Most lncRNAs are located in the nucleus, in which they work as scaffolds for chromatin modifiers or transcriptional co-regulators to exhibit regulatory functions (184). In addition, lncRNAs can alter transcription, mRNA stability, alternative splicing, and translational activity in AD, resulting in aberrant gene expression (14). Overall, the deregulated and complex non-coding RNAs are closely associated with core pathophysiological processes of AD via regulating gene expression at different levels, including transcription, RNA processing, and translation (185).

EPIGENETIC THERAPEUTIC STRATEGIES FOR AD

DNA hypomethylation of pathogenetic genes is associated with the overproduction of Aβ (186). Oliveira et al. find that DNA methyltransferase Dnmt3a2 is decreased in the hippocampus of mice, and the restoration of Dnmt3a2 recovered the cognitive functions (160, 187). Further, betaine, a methyl donor, ameliorates the memory deficits in mice (161). Another methyl donor, S-adenosylmethionine (SAM), is decreased in the cerebrospinal fluid of AD patients (188). SAM reduces the production of Aβ and tau phosphorylation by upregulating PSEN1 and BACE1 expression *in vivo*, which improves the cognitive status in AD mouse models (162). The treatment with alcohol extracts from G. lucidum increases methylation regulators and improves memory in APP/PS1 AD model mice (163). In AD and mild cognitive impairment patients, B vitamin intake results in hypermethylation of *NUDT15* and *TXNRD1,* which is associated with better cognitive performance (164). The supplementation of folic acid, a methyl donor, improves cognitive functions in participants who tend to decline with age (165). Interestingly, maternal supplementation of resveratrol promotes cognitive decline in the SAMP8 mice offspring via increasing global methylation levels and decreasing hydroxymethylation levels (166). Consequently, the DNA methyltransferase or the methyl donor may be potential treatments for patients with AD.

Meanwhile, hypermethylation is involved in the development of AD as well; thus, the decrease of methylation levels in some genes may also be a promising therapeutic strategy (109). DNMT inhibitors are used in the treatment of hematopoietic malignancy (189). Also, the administration of DNMT inhibitors is used in some neurodegenerative diseases, such as Friedreich's ataxia (190). Although DNMT inhibitors possess the potential for the therapy of AD, the lack of gene-specificity and security is the main difficulty needed to resolve before its use in AD patients (177). As we previously mentioned, the epigenetic markers are altered in the 5xFAD mouse model. Treatment of UNC0642 inhibits the methyltransferase activity G9a/GLP and restores cognition by reducing 5mC and increasing 5hmC in the 5xFAD mouse model (167). The knockout of the *Tet1* gene enhances

TABLE 4 | Non-coding RNAs in AD.

Non-coding RNAs	Tissue type	Main findings	References
MicroRNA-29a/b-1	AD patients postmortem sporadic brain	*MicroRNA-29a/b-1* could suppress BACE1 expression and was significantly decreased in AD	(112)
MicroRNA-107	Human temporal cortex samples	Decreased *microRNA-107* expression	(116)
MicroRNA-298	Primary human cell culture model	*MicroRNA-298* repressed the expression of BACE1, APP, Aβ40, and Aβ42	(120)
MicroRNA-34a-5p and *microRNA-125b-5p*	Serum samples of 27 AD patients	*MicroRNA-34a-5p* and *microRNA-125b-5p* reduced Aβ	(121)
MicroRNA-125b	Primary neurons	*MicroRNA-125b* caused tau hyperphosphorylation	(124)
MicroRNA-132	Primary mouse and human wild-type neurons	*MicroRNA-132* was associated with the lower levels of tau phosphorylation	(126)
MicroRNA−219-5p	Human postmortem brain tissues	*MicroRNA-219-5p* was increased and associated with tau phosphorylation in AD	(122)
MicroRNA-125b	Human postmortem brain specimens	*MicroRNA-125b* could promote the phosphorylation of tau and was enhanced in AD	(123)
MicroRNA-346	Primary human brain cultures	*MicroRNA-346* upregulated APP translation and Aβ production	(132)
MicroRNA-644	Human HEK293, HeLa cells, and mouse Neuro2A cells	*MicroRNA-644* downregulated the formation of Aβ	(133)
circRNA	Neuropathologically confirmed AD case and control brain tissues	CircRNA was associated with AD diagnosis, clinical dementia severity and neuropathological severity	(137)
Circ-AXL, *circ-GPHN* and *circ-PCCA*	Cerebrospinal fluid from AD patients and control subjects	*Circ-AXL*, *circ-GPHN* and *circ-PCCA* differed significantly between AD patients and normal controls	(141)
circRNA	Human postmortem brain samples	147 circRNAs were differentially expressed in different AD brain regions	(142)
BACE1-AS	Human postmortem brain samples	BACE1-AS could increase BACE1 mRNA stability and generate additional Aβ	(144)
51A, 17A, and NDM29	Postmortem AD brain samples and AD cerebrospinal fluid	17A, 51A, and NDM29 increase Aβ formation and/or the $A\beta_{42}/A\beta_{40}$ ratio	(147)
LncRNAs	Human postmortem brain samples	*SNHG19* and *LINC00672*, *RNF144A-AS1*, *LY86-AS1*, and *LINC00639* were associated with the pathology of AD	(156)
BC200	Human postmortem brain samples	*BC200* levels enhanced significantly in AD brains	(149)
BDNF-AS	Human and mouse cell lines	*BDNF-AS* repressed BDNF expression	(150)
Sox2OT	AD mouse cerebral cortex	*Sox2OT* repressed *Sox2* gene expression, involving in neurogenesis and neuronal differentiation	(151)
EBF3-AS	Hippocampus of APP/PS1 mice	*EBF3-AS* was upregulated in hippocampus and related to neuron apoptosis	(152)
NAT-Rad18	AD rat cortical neurons	*NAT-Rad18* increased the likelihood of apoptosis	(153)
TUG1	AD mice model	*TUG1* promoted neuronal apoptosis	(154)
LncRNAs	SAMP8 mice	3,112 differentially expressed lncRNAs were found in hippocampus	(155)
NEAT1	APPswe/PS1dE9 double transgenic mouse model	*NEAT1* is involved in Aβ clearance	(158)
NEAT1	APP/PS1 transgenic mice model	*NEAT1* promoted the pathogenesis of AD	(159)
MicroRNA-124-3p	N2a/APP695swe cells	*MicroRNA-124-3p* inhibited abnormal tau hyperphosphorylation	(127)
MicroRNA-106b	SH-SY5Y cells	*MicroRNA-106b* reduced tau phosphorylation	(128)
MicroRNA-330	C57 mice	*MicroRNA-330* reduced Aβ production and alleviated oxidative stress	(134)
MicroRNA-138	APP/PS1 mice	*MicroRNA-138* enhanced Aβ production and improved cognitive impairment	(135)
MicroRNA-98	AD mice model	*MicroRNA-98* improved oxidative stress and downregulated Aβ production	(136)
MicroRNA-369	3xTg-AD mice	*MicroRNA-369* was associated with tau hyperphosphorylation	(129)
MicroRNA-425-5p	AD and HEK293/tau cells	*MicroRNA-425-5p* overexpression promoted tau phosphorylation	(125)

(Continued)

TABLE 4 | Continued

Non-coding RNAs	Tissue type	Main findings	References
MicroRNA-326	AD mice models	*MicroRNA-326* decreased tau hyperphosphorylation and improved cognitive functions	(130)
MicroRNA-195	ApoE4$^{+/+}$ mice	*MicroRNA-195* diminished tau hyperphosphorylation and Aβ burden	(131)
MicroRNA-31	Hippocampus of 17-month-old AD triple-transgenic (3xTg-AD) female mice	*MicroRNA-31* was able to reduce the mRNA levels of BACE1	(115)
MicroRNA-200a-3p	Hippocampus of APP/PS1 and SAMP8 mice as well as in blood plasma from AD patients	*MicroRNA-200a-3p* could reduce the expression of BACE1 and confirmed to be decreased in AD	(113)
MicroRNA-16	Hippocampus of aged SAMP8 mice and murine cells	*MicroRNA-16* led to reduced APP protein expression and was decreased in AD mice	(111)
MicroRNA-340	Hippocampus of AD model SAMP8 mouse	*MicroRNA-340* was decreased and associated with the overproduction of Aβ	(114)
BACE1-AS	SAMP8 mice	Knockdown of BACE1-AS inhibited BACE1 and improved memory	(145)
SOX21-AS1	AD mice model	LncRNA SOX21-AS1 was unregulated and resulted inneuronal oxidative stress in AD	(148)
MicroRNA-195	SAMP8 mice and HEK293 cells	SAMP8 mice and HEK293 cells	(119)
MicroRNA-124	Cellular AD model	*MicroRNA-124* was steadily altered and associated with BACE1	(117)
MicroRNA-298 and *microRNA-328*	Neuronal (N2a) and fibroblastic (NIH 3T3) cells	*MicroRNA-298* and *microRNA-328* were associated with BACE1	(118)
circRNA *circ_0000950*	Cellular AD model	*Circ_0000950* promoted neuron apoptosis, inhibited the production of neurite outgrowth, and increased the levels of inflammatory cytokines levels	(139)
circRNA *CircNF1-419*	SD rat model	*CircNF1-419* upregulated autophagy and reduced Aβ and tau	(140)

circRNA, circular RNA; BACE1-AS, BACE1-antisense transcript; SOX21-AS1, SOX21 antisense RNA 1; NEAT1, Nuclear paraspeckle assembly transcript 1.

cognitive function by oxidizing 5 mC to 5 hmC and reducing methylation levels of the brain in mice (191).

Histone deacetylases (HDACs) inhibit gene expression and are associated with memory impairment by restricting access of transcription factors to memory storage-related genes (192). HDAC inhibitors (HDACi) are considered to be the potential therapeutic strategy in AD. Trichostatin A, an HDACi, restores contextual freezing performance and H4 acetylation levels in the APP/PS1 mouse model of AD (100). Valproic acid (VPA) is one of the first discovered HDACi and beneficial in memory enhancement in the mouse model of AD (168). Histone deacetylase 2 (HDAC2) inhibitors improve memory by promoting the formation and growth of dendritic spines in mice (193, 194). Sodium phenylbutyrate, an HDACi, also alleviates memory impairment in transgenic AD mice by inducing neurotrophin expression via the protein kinase C (PKC)-cAMP-response element-binding protein (CREB) pathway (169). M344, an HDACi, lowers the expression of Aβ and prevents cognitive decline with the normalization of several pathogenic pathways in triple transgenic (APPsw/PS1M146V/TauP301L) mice *in vivo* (170). A mercaptoacetamide-based class II HDACi and a hydroxamide-based class I and II HDACi reduce Aβ levels *in vitro* and rescue memory loss in AD mice (171). The downregulated PU.1 expression is associated with lower AD risk in a genome-wide association study (195). High-throughput screening of FDA-approved drugs reveals a HDACi, vorinostat, decreases PU.1 expression and may be a useful therapeutic approach in AD (172). Moreover, the administration of RGFP-966, a selective HDAC3 inhibitor, improves cognitive function in the AD mouse model and decreases Aβ and tau in neurons from AD patients, further supporting the significant role of HDACi in patients beyond the AD mouse model (173). Therefore, HDACi can be seen as a potential therapeutic agent for AD. However, HDACi is widely targeted and inevitably causes various side effects, including apoptosis and arrest of the cell cycle; therefore, successful experiments in AD animal models are seldom feasible for clinical application in humans. To increase the sensitivity of HDACi is a critical issue in the future (196). Moreover, histone acetyltransferase (HAT) is involved in the formation of CREB binding protein (CBP), which has an important role in memory (197). The expression of CBP could help transgenic AD mice recover memory impairment. The activator of histone acetyltransferases CBP/p300 is capable of passing the blood–brain barrier and extending the recent memory duration in B57BL6/6J male mice *in vivo*, which may be a potential treatment target in AD (174). In the late-stage FAD mouse model, the inhibitors of euchromatic histone methyltransferases decrease histone hypermethylation and improve synaptic deficits and cognitive functions, providing a possible novel therapeutic strategy for AD (175).

Noncoding RNAs are also involved in the pathogenesis of AD. The decreased expression of BACE1 is obtained by

TABLE 5 | Epigenetic therapeutic strategies in AD.

Therapeutic strategies	Model	Main findings	References
Dnmt3a2 (DNA methyltransferase)	Aged mice	The raise Dnmt3a2 level in the hippocampus of aged mice enhanced cognitive ability	(160)
Betaine(a methyl donor)	Male ddY strain mice	Betaine treatment ameliorate memory deficit	(161)
S-adenosylmethionine(SAM, a methyl donor)	TgCRND8 mice	SAM reduced the Aβ production and improved the memory	(162)
Alcohol extracts from G. lucidum	APP/PS1 AD model mice	It increased methylation regulators and improved memory	(163)
B vitamin	AD patients and mild cognitive impairment patients	It resulted in hypermethylation of *NUDT15* and *TXNRD1* and better cognitive performance	(164)
Folic acid	Participants who tend to decline with age	The supplementation of folic acid improved cognitive functions	(165)
Resveratrol	SAMP8 mice	It promoted cognitive decline in the SAMP8 mice offspring	(166)
UNC0642	5XFAD mouse model	It inhibited the methyltransferase activity G9a/GLP and restored cognition	(167)
Trichostatin A	AD mouse model	It restored contextual freezing performance	(100)
Valproic acid (VPA, a histone deacetylase inhibitor)	APPswe/PS1ΔE9 (APP/PS1) transgenic mice	VPA decreased Aβ deposition and increased memory ability	(168)
Sodium phenylbutyrate (a histone deacetylase inhibitor)	5XFAD mice	Sodium phenylbutyrate improved memory and spatial learning	(169)
M344 (a histone deacetylase inhibitor)	Triple transgenic (APPsw/PS1M146V/TauP301L) mice	M344 lowered the expression of Aβ and prevent cognitive decline	(170)
Mercaptoacetamide-based class II HDACi and a hydroxamide-based class I and II HDACi	3xTg AD mice	They reduced Aβ levels *in vitro* and rescued memory loss	(171)
Vorinostat	Primary human brain tissue	It decreased PU.1 expression and was associated with lower AD risk	(172)
RGFP-966	AD mice model	It improved cognitive functions	(173)
CBP/p300 (an activator of histone acetyltransferase)	3xTg-AD mice	CBP/p300 extended the recent memory duration	(174)
The inhibitors of euchromatic histone methyltransferases	FAD mouse model	It decreased histone hyper-methylation and cognitive functions	(175)
Short-interfering RNA	AD mice models	It decreased the expression of BACE1	(176)
RNA interference	AD mice models	It downregulated the expression of APP, PSEN1, PSEN2	(177)
MicroRNA-384 mimic	SH-SY5Y cells	*MicroRNA-384* mimic downregulated the expression of APP and BACE1	(178)
Anti-microRNA-146a-base treatment	AD mouse model	It improved cognitive functions in AD	(179)
Inhibitor of *microRNA-34c*	AD mice models	It could enhance the memory ability	(180)
Knockdown of BACE1-AS by lentivirus	SAMP8 mice	It improved learning behaviors and memory	(145)
MicroRNA-124	SH-SY5Y cells	It decreased apoptosis and decreased Aβ-induced viability inhibition	(181)

HDACi, inhibitor of histone deacetylase; BACE1-AS, BACE1-antisense transcript; NUDT15, nudix hydrolase 15; TXNRD1, thioredoxin reductase 1.

short-interfering RNA, resulting in the reduction of Aβ and tau phosphorylation levels in AD transgenic mice (176). The expression of APP, PSEN1, and PSEN2 was downregulated by RNA interference, such as short-interfering RNA and short hairpin RNA, which provide a promising therapeutic strategy in the future (177). MicroRNA mimics and anti-microRNAs are being developed by decreasing target protein expression in AD. *MicroRNA-384* mimic downregulating the expression of APP and BACE1 in SH-SY5Y cells, demonstrating that *microRNA-384* may be a potential target in AD (178). Anti-microRNAs complement respective microRNAs and reduce their levels to restore homeostasis. In the AD mouse model, *microRNA-146a* is upregulated, and anti-microRNA-146a-base treatment improves cognitive functions and regulates the inflammatory response by using the viral vector delivery system (179). In addition, a number of other microRNAs are the potential treatment targets in AD. *MicroRNA-34c* increases in the hippocampus and blood of patients with AD, and its

inhibitor enhances memory in AD mice models *in vivo* (180). LncRNA *BACE1-AS* is positively associated with BACE1 protein expression *in vitro* and *in vivo*, and knockdown of *BACE1-AS* by short interfering RNA improves cognitive function in a mouse model of AD (145). *MicroRNA-339-5p*, *microRNA-29c*, and *microRNA-124* decrease BACE1 expression *in vitro* (181, 198, 199) (**Table 5**). *MicroRNA-101* suppresses APP and Aβ expression in hippocampal neurons *in vitro* (200). *MicroRNA-153* decreases APP expression in primary human fetal brain cultures (201). Consequently, non-coding RNAs may be potential targets for AD therapy in the future. Currently, there are several problems in the treatment of AD through non-coding RNAs, including altering such targets, off-target effects, and delivery methods (202). However, it is worthwhile to investigate non-coding RNAs in AD by appropriate understanding and safe manipulation (203).

Currently, a few drugs are available for the treatment of AD. No drug can cure or stop disease development. Given the huge number and seriousness of AD, the need for clinical trials is necessary. To date, most of the clinical trials of AD have targeted Aβ, tau protein (204). Several clinical trials investigate epigenetics for the treatment of AD. Oral betaine was given in eight AD patients; however, the efficacy of betaine could not be determined due to the lack of controls and small sample size (205). With the use of S-adenosylmethionine and nutriceutical, almost 30% improvement in the neuropsychiatric inventory and activities of daily living is observed in AD patients compared to normal controls (206). RDN-929, a selective HDAC inhibitor, is being investigated in a phase I clinical trial for the treatment of AD patients. The result is not disclosed at clinicaltrials.gov. EVP-0334, also called FRM-0334, a CNS-penetrant HDACi, phase I testing of AD is completed; however, no result is posted (207). Because increasing evidence shows that epigenetics plays an essential role in AD, the drugs related to epigenetics may be breakthroughs for AD in the future.

CONCLUSION

To date, although some genetic and non-genetic factors are well-studied, the pathogenesis of AD remains unclear. Epigenetics provides us with an important insight into how AD develops. There is an increasing number of studies about epigenetics in AD patients, including DNA methylation/hydroxymethylation, histone modifications, and non-coding RNAs. Epigenetic genome-wide association (EGWA) studies show that many differentially methylated sites exist in AD compared with normal controls. Several studies investigate the role of histone modifications in AD. Non-coding RNAs play an important role in the pathogenesis of AD. LncRNAs, such as *BACE1-AS*, increases BACE1 mRNA stability and generates additional Aβ in AD. These studies show us that epigenetics is of great importance in AD, suggesting that epigenetics can be a potential intervention target in treating AD given the reversible nature of epigenetic changes. Therapeutic attempts include the use of inhibitors of HDACs, DNA methyltransferase, and inhibitors of non-coding RNAs, which have shown some exciting results in animal studies. Despite the numerous and exciting findings of epigenetics in AD, the results are less satisfying. The data is often controversial and lacks definite results. There is a need to design some larger longitudinal cohorts to study the epigenetic changes of AD, which may help us better understand the pathogenesis of AD and find novel strategies to treat AD in the future.

AUTHOR CONTRIBUTIONS

BJ was involved in the review design, modified, and revised the manuscript. XX and XL searched and reviewed the articles. XX wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Sporadic Creutzfeldt-Jakob Disease and Other Proteinopathies in Comorbidity

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Background: Sporadic Creutzfeldt–Jakob disease (sCJD) is the most common type of a group of transmissible spongiform encephalopathies (prion diseases). The etiology of the sporadic form of CJD is still unclear. sCJD can occur in combination with other neurodegenerative diseases, which further complicates the diagnosis. Alzheimer's disease (AD), e.g., is often seen in conjunction with sCJD.

Method: In this study, we performed a systematic analysis of 15 genes related to the most important neurodegenerative diseases - AD, frontotemporal dementia, amyotrophic lateral sclerosis, prion disease, and Parkinson's disease - in a cohort of sCJD and sCJD in comorbidity with AD and primary age-related proteinopathy (PART). A total of 30 neuropathologically verified cases of sCJD with and without additional proteinopathies were included in the study. In addition, we compared microtubule-associated protein tau *(MAPT)* haplotypes between sCJD patients and patients with sCJD and PART or sCJD and AD. Then we studied the interaction between the Apolipoprotein E gene (*APOE)* and *PRNP* in sCJD patients.

Results: We did not find any causal mutations in the neurodegenerative disease genes. We did detect a p.E318G missense variant of uncertain significance (VUS) in *PSEN1* in three patients. In *PRNP*, we also found a previously described non-pathogenic insertion (p.P84_Q91Q).

Conclusion: Our pilot study failed to find any critical differences between pure sCJD and sCJD in conjunction with other comorbid neurodegenerative diseases. Further investigations are needed to better understand this phenomenon.

Keywords: Creutzfeldt-Jakob disease, Alzheimer's disease, β amyloid, tau protein, neurodegenerative disease

INTRODUCTION

Neurodegenerative diseases are characterized by intra-or extracellular accumulation of specific protein aggregates in the central nervous system (CNS) (1). These proteins have a predominantly β-sheet form and are found in a number of neurodegenerative diseases such as Alzheimer's disease (AD); synucleinopathies (Parkinson's disease (PD), multiple system atrophy, dementia with Lewy bodies); transmissible spongiform encephalopathies (TSE; also known as prion disease); amyotrophic lateral sclerosis and frontotemporal dementia (2). There is a significant overlap of symptoms resulting from the multiplication and tissue storage of protein aggregates in the brain, leading to progressive neuronal dysfunction and neurodegeneration (3, 4).

Creutzfeldt-Jakob disease (CJD; MIM #176640), the most common human prion disease with an estimated incidence of 2 cases per million per year, is comprised of several clinical-pathological phenotypes and occurs in four unique forms (sporadic, genetic, variant, or acquired), each with seemingly distinct etiologies (5).

CJD can coexist with other neurodegenerative diseases because the presence of both Aβ and tau pathology is not unusual in sporadic and genetic CJD brains (6–9). Primary age-related proteinopathy (PART) is a common pathology involving misfolded tau protein aggregates associated with human aging (10). PART can cause cognitive impairment in the absence of AD (11); additionally, the coexistence of PART and sporadic CJD (sCJD) has been reported (12). A major genetic risk factor for PART is the haplotype of the microtubule-associated protein tau (*MAPT*) (13). The frequency of Apolipoprotein E (APOE) ε4 is much lower in PART, being ∼10% (10, 14), whereas its prevalence in AD exceeds 45% (15, 16). These studies suggest that APOE ε4 allele deficiency – in contrast to AD – is not a risk factor for PART.

Coexistence with other neurodegenerations is relatively common in sCJD patients. Since clinical symptoms of sCJD can overlap with manifestations of other comorbid disorders, establishing a clinical diagnosis in patients with rapidly progressive dementia is very difficult (17), and a definite diagnosis can only be made after a neuropathological examination of the brain.

Our goal was to identify disease-associated variants using genetic studies of sCJD patients. For this reason, we compared sCJD patients without any comorbid proteinopathies to sCJD patients with AD and sCJD patients with PART.

MATERIALS AND METHODS

Study Population

Our study was designed as a retrospective study. We included patients with post-mortem confirmed sCJD, as well as information regarding clinical presentation and data from neuropsychological testing, biochemical analysis, EEG, and neuroimaging. Neuropathological diagnoses, including prion protein immunoassays, were provided according to standard protocols National CJD Research & Surveillance Unit. Protocol: Surveillance of CJD in the UK) (18) used by the National reference laboratory for human prion diseases at the Department of Pathology and Molecular Medicine, Prague, Czech Republic. Molecular genetic analyses were performed in the Neurodegenerative Brain Disease group of the VIB Center for Molecular Neurology, Antwerp, Belgium.

We divided our cohort into three subgroups: (1) isolated sCJD neuropathology, (2) sCJD and PART or early stage AD (NIA consensus criteria level "low") (19), and (3) sCJD with more advanced AD (NIA consensus criteria level A2 and or higher).

The Molecular Diagnostics Study Group

All autopsied patients (30/30) fulfilled the WHO diagnostic criteria for definite sporadic CJD (18)[1] and were genetically profiled for the most common genes ($n = 15$) associated with AD (*APP, PSEN1, PSEN2, APOE*), the FTD-ALS spectrum (*MAPT, GRN, TARDBP, FUS, SOD1, VCP*), prion disease *(PRNP*), and PD (*LRRK2, PRKN, SNCA*) (**Supplementary Table 1**).

Other available clinical data, which were designated as variables (including age at onset, age at death, gender, and symptoms occurring during the disease), were analyzed to determine how they affected the pathogenesis of sCJD and the concomitant Aβ and tau pathologies.

Genetic Screen

Mutation analyses by gene panel sequencing were performed on genomic DNA extracted from bone marrow. The targeted gene panel captured all exons of the 15 genes and flanking intronic regions to cover the splice sites. Using amplicon target amplification technology (Agilent, https://www.agilent.com), primers were designed using mPCR software (20) (**Supplementary Material**). Specific target regions were amplified using multiplex PCR, followed by purification of the equimolar pooled amplicons using Agencourt AMPureXP beads (Beckman Coulter, CA, USA). Individual barcodes (Illumina Nextera XT) were incorporated in a universal PCR step prior to sample pooling. Libraries were sequenced on a MiSeq platform using the v3 reagent kit with a paired-end read length of 300 bp (Illumina, San Diego, CA, USA). Non-sense, splice site, indel, and missense variants, with a minor allele frequency (MAF) $\leq$ 1%, were selected.

Results

We analyzed data from 30 patients ($n = 30$) with a mean age at onset (AAO) of 58.4 $\pm$ 5 years, and a male-to-female ratio of 18:12. Ten cases had sCJD without any other comorbid proteinopathy, 10 cases had sCJD with tauopathy and/or early evolved AD, and 10 cases had sCJD with more developed AD. Family histories were available in 24 cases (82%), with only one patient (3.4%) having a positive family history for dementia. No family histories of CJD were reported. Effects of rare (MAF $\leq$ 1%) missense variants on protein structure and function were predicted using SIFT (http://sift.jcvi.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and

[1] www.cjdsupport.org/, https://www.cjdsupport.org.au/site/wp-content/uploads/2017/04/PRNP-Guidelines-160417.pdf

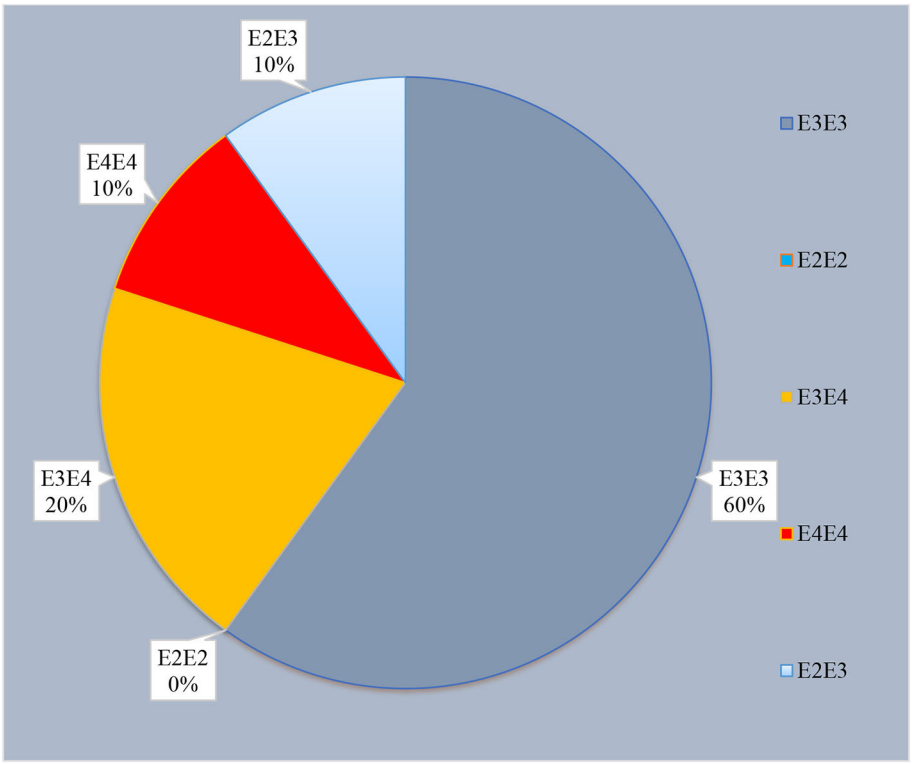

FIGURE 1 | Distribution of *APOE* polymorphism carriers in our cohort ($n = 30$).

SNP&GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/) (**Supplementary Table 2**).

Clinical manifestations included mild to moderate dementia with predominant executive and speech/language impairment (aphasia, dysarthria) with less impaired memory and visuospatial function. Behavioral and psychiatric manifestations (depression, apathy, irritability, anxiety, aggression, visual hallucinations, and insomnia) were described in most patients. Motor symptoms typically included Parkinsonism, spasticity, gait disturbance, and/or immobility (**Supplementary Table 3**).

Mutation Screening

Gene panel screening (15 genes) for variants and mutations associated with AD, FTD-ALS, and PD, revealed 4 rare, protein-modifying variants (**Supplementary Table 2**). Effects on protein structure and function were predicted using SIFT (http://sift.jcvi.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and SNP&GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/). In *PSEN1*, the p.E318G polymorphism in *PSEN1* was found in three patients (10.3%), of which one had pure sCJD, and two were sCJD + AD. No relevant variants were observed in the other AD genes *APP* and *PSEN2*. In *PRNP*, the p.P84_Q91Q insertion was detected in one patient with β-amyloidopathy. This variant is considered non-pathogenic (21). Furthermore, one benign missense variant was present in *GRN* and one in *SOD1*. We did not find any potential disease-causing mutations in the PD genes *SNCA*, *LRRK2,* and *PRKN;* silent mutations were found (**Supplementary Table 2**).

Genetic Predisposing Factors - ε4 Allele of Apolipoprotein *E* (*APOE*)

APOE polymorphic variants were tested at codons 112 and 158. Of the 30 cases in our study, 10% ($n = 3$) carried the ε4 allele of the *APOE* gene (**Figure 1**). All three cases were AD level A2 (AAO 62, 75, 83 years). The distribution of the polymorphic codon 129 of *PRNP* and *APO* genotypes in sCJD patients are shown in **Supplementary Table 4**. We found no association between *APOE* ε4 allele status and sCJD; however, the *APOE* ε4 was seen in two *PRNP* M129M homozygotes ($n = 2$).

MAPT Haplotype Association With Sporadic CJD

We analyzed MAPT haplotypes in both isolated sCJD cases and in cases with sCJD and tauopathy. We identified only one case with the H2/H2 haplotype, and they were in the comorbid subgroup (**Supplementary Table 5**). As such, our study shows no evidence of an association between MAPT gene variations and sCJD, which could have contributed to the tau deposits in the CNS.

DISCUSSION

In our study of 30 cases of sCJD in the Czech Republic (the annual rate of definite CJD is about 20 cases/yr.), we analyzed the most important genes related to neurodegeneration. The cognitive profile in our patients was characterized by a

heterogeneous manifestation, with predominant involvement of executive and speech/language functions with a significant proportion also having behavioral manifestations (including visual hallucinations).

We did not detect any pathogenic mutations in the *PRNP* gene. Our study also tried to determine if there were any predisposing genetic factors that could account for the occurrence of comorbid Aβ and tau protein deposits in CJD brains. Previous studies have provided evidence that comorbid proteinopathy is not unusual in CJD brains, although the exact mechanism by which β-amyloid and tau deposits spread within brain tissue remains unclear (22). Since several studies have documented a possible spread of β-amyloid in brain tissue (23, 24), we performed a mutation analysis of *APP* (Aβ encoding exons) as well as the coding region of *PSEN1* and *PSEN2*. However, we did not find any mutations in the genes that would explain the increased Aβ42 production.

There is only sparse evidence supporting the potential interaction between *APOE* and *PRNP* in sCJD. Recent studies that analyzed the influence of *APOE* on CJD have yielded discordant results. Three of our cases had the *APOE* ε4 genotype (AAO > 70 years on average), i.e., β-amyloidopathy level A2 and Methionine/Methionine homozygosity at codon 129 of the *PRNP* gene (M129M). Recent studies have suggested variants of PRNP129 (methionine/methionine, methionine/valine, valine/valine) as possible modifiers of AD disease (25). However, because of the small sample size of our study, this interpretation should be approached cautiously. Further studies should be carried out to assess the effects of PRNP129 in the AD phenotype. We found no influence of the *APOE* genotype relative to the age at onset, nor any significant differences in the distribution of the *APOE* ε4 and ε2 genotypes relative to those with isolated sCJD and those with sCJD and AD. Our results are consistent with other studies showing that *APOE* is not a risk factor for CJD (26–29).

The pathology of tau in sCJD brains is not unique, and in our cohort, this additional pathology was seen in 6 of the 30 definite CJD patients (30%) (6). Tau is encoded by the *MAPT* gene, and there are two common *MAPT* extended haplotypes, i.e., H1 and H2 (29). Only one study has investigated the role of MAPT in the etiology of sCJD (30). There is somewhat more evidence regarding the role of MAPT haplotypes (H1 and H2) in neurodegenerative diseases. H1 has been linked to FTLD and AD (31), whereas H2 is associated with a lower risk for developing late-onset AD (32). Our study shows no evidence for any association between *MAPT* gene haplotypes and sCJD.

The coexistence of CJD and PD is exceedingly rare. Several reported case studies show that α-synuclein amyloid deposits in CJD patients are associated with a slower disease course. The precise molecular mechanism explaining how misfolded α-synuclein accumulates and spreads in synucleinopathies is still unknown (33). Sequence or copy number variants in at least six genes (*SNCA, LRRK2, PRKN, PINK1, DJ-1,* and *ATP13A2*) have been identified to cause monogenic forms of PD (34). To date, no mutations responsible for PD have been reported in patients with CJD. Due to the low incidence of patients with proven CJD and PD, it is not clear whether there are gene interactions between CJD and PD. Our study, however, was not focused on the issue of sCJD and synucleinopathy, due to the extremely low incidence of both pathologies in comorbidity. This issue is, nevertheless, a promising direction for future research, and as such, it could help us better understand the genetic background as well as perhaps offer novel therapeutic options.

In conclusion, we failed to find any association between the investigated genes and the accumulation of specific protein aggregates in the examined brain tissue. These findings suggest that comorbid neurodegenerative disorders in sCJD behave as if they were independent processes taking place within the same brain; additionally, the underlying pathophysiology of comorbid protein deposits in CJD appears to have a complex multifactorial origin.

It would, however, be promising in the future to examine other risk genes for AD, FTD, and PD, and their potential association with CJD (**Supplementary Table 6**) (35). The search for genetic evidence of clinical, pathological, and possible molecular overlap between neurodegenerative diseases certainly needs to continue and would be best done with a larger multicenter cohort.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: This manuscript utilizes proprietary data. Requests to access these datasets should be directed to Julie van der Zee, julie.vanderzee@uantwerpen.vib.be, Neurodegenerative Brain Diseases Group, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium. Primers for the targeted assay were designed using proprietary mPCR software from Agilent (previously Multiplicom) (20).

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JZ created the concept and design of the project. CVB provided laboratory facilities for the practical implementation of the entire project, provided complete instrumentation of the VIB Center for Molecular Neurology. JZ and CVB critically revised the manuscript. LD provided recommendations and specific approaches to the samples analysis (analysis tools) as an expert technician in the laboratory. RR diagnosed in detail the neurological cases mentioned in the article. RM verified the neuropathological diagnosis of neurodegenerations

and approved the final version to be published. All authors participated in the manuscript.

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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.2020.596108/full#supplementary-material

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