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Disease progression model in subjects with mild cognitive impairment from the Alzheimer's disease neuroimaging initiative: CSF biomarkers predict population subtypes

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Amnestic mild cognitive impairment MCI) represents the prodromal stage of Alzheimer's dementia and this disease progresses in a non-linear fashion.
- Disease progression depends on a variety of demographic, biochemical, genetic and cognitive factors.

WHAT THIS STUDY ADDS

- Baseline CSF biomarkers carry information about disease pathology and critical thresholds for these markers (Aβ and p-tau$_{181P}$) have been identified that allow segregation of the population into MCI progressers and non-progressers.

AIM

The objective is to develop a semi-mechanistic disease progression model for mild cognitive impairment (MCI) subjects. The model aims to describe the longitudinal progression of ADAS-cog scores from the Alzheimer's disease neuroimaging initiative trial that had data from 198 MCI subjects with cerebrospinal fluid (CSF) information who were followed for 3 years.

METHOD

Various covariates were tested on disease progression parameters and these variables fell into six categories: imaging volumetrics, biochemical, genetic, demographic, cognitive tests and CSF biomarkers.

RESULTS

CSF biomarkers were associated with both baseline disease score and disease progression rate in subjects with MCI. Baseline disease score was also correlated with atrophy measured using hippocampal volume. Progression rate was also predicted by executive functioning as measured by the Trail *B*-test.

CONCLUSION

CSF biomarkers have the ability to discriminate MCI subjects into sub-populations that exhibit markedly different rates of disease progression on the ADAS-cog scale. These biomarkers can therefore be utilized for designing clinical trials enriched with subjects that carry the underlying disease pathology.

Introduction

It is believed that by the time Alzheimer's disease (AD) is diagnosed, sufficient neuronal injury has occurred that reversal of the disease is perhaps unlikely [1]. This has therefore raised considerable interest in the prodromal stage of AD involving subjects with mild cognitive impairment (MCI) who are in the pre-dementia stage of cognitive dysfunction and therefore could be targeted for therapies that could potentially provide beneficial effects. The prevalence rate for MCI around the world is in the range of 14–18% in individuals $\geq$ 70 years of age [2]. In clinical trials and epidemiologic studies the annual rate of conversion of MCI subjects to dementia is in the range of 6–15%, which is much higher than the incidence rate of dementia of 1–2% seen in the general population [2]. MCI represents an intermediate state of cognitive impairment that is greater than the level expected for a subject's education level and age [3] but does not meet criteria for dementia and does not compromise activities of daily living. The diagnosis of MCI is characterized by heterogeneity, varying severity and the inability to predict disease progression i.e. not all MCI subjects have underlying AD neuropathology [2]. Indeed, not all cases of MCI progress to AD and a small fraction of subjects revert back to normal status. However, the clinical phenotype of amnestic MCI, in which only the domain of memory is affected, is thought to be degenerative in nature and these subjects have a high probability of progression to AD [1].

Neuropsychological assessments are a key component of detecting and tracking disease progression in clinical trials because they provide standardized evaluation of memory and cognitive impairments which are central features of MCI. The cognitive component of the AD Assessment Scale (ADAS-cog) has been utilized in the majority of large scale pharmacologic and naturalistic studies of MCI. During the past decade several MCI clinical trials have tested the current pharmacologic agents used in the treatment of AD. None of these clinical trials has achieved their expected therapeutic end points and therefore there are no approved treatments for MCI [2]. These results have caused some concerns about the insufficient sensitivity of the ADAS-cog scale in mapping and tracking the early stages of the disease [4].

From the published CSF biomarkers total tau, phosphorylated tau at the threonine 181 position (p-tau$_{181p}$), and CSF amyloid beta 1 to 42 peptide (Aβ1–42) are considered as promising markers for inclusion in clinical trials and in the revised AD diagnostic criteria [5, 6]. The utility of these biomarkers is further supported by the newly released National Institute on Aging/Alzheimer's Association Diagnostic Guidelines for AD that recommend inclusion of these specific markers for use in research settings, including MCI clinical trials [7]. Recent reports from the AD neuroimaging initiative (ADNI) trial have shown that MCI subjects exhibit bimodal distributions with respect to their baseline concentrations of Aβ1–42 and p-tau$_{181P}$ [5]. These biomarkers (low CSF Aβ1–42 and high p-tau$_{181P}$) are thought to reflect the pathologic features associated with AD. CSF biomarkers have the potential to provide information about the probability of disease progression to AD for an individual MCI patient and the likelihood that this progression will occur within a defined period. The CSF biomarkers considered in the current analysis represent a small subset of the large number of related biomarkers [6]. However, the CSF biomarkers have a fairly large body of literature evidence in MCI [8–12] and are therefore considered as a reasonable starting point. CSF biomarkers were captured in only 50% of subjects in the ADNI trial and the current analysis will focus on only those ADNI MCI subjects who have baseline CSF data available for Aβ1–42 and tau proteins.

A disease progression model was previously developed for patients with AD [13, 14] and since the MCI population represents a distinctly different sub-group in terms of biomarker characteristics and rates of cognitive deterioration [5], the current analysis focuses on this earlier stage of the disease. The recently developed semi-mechanistic non-linear AD disease progression model was built to (a) capture the longitudinal change of ADAS-cog scores and (b) describe the rate of progression and baseline ADAS-cog as a function of influential covariates in AD patients [13, 14]. In the model, baseline ADAS-cog was associated with years since dementia onset, hippocampal volume and ventricular volume. Disease progression rate was dependent on age, total serum cholesterol, APOE ε4 (APOE4) genotype, Trail B test, as well as current impairment status measured by ADAS-cog. Rate of progression was slower for mild and severe AD patients *vs*. moderate AD patients who exhibited a faster rate of disease worsening. One of the objectives of the current analysis is to assess the applicability of this AD model and its covariate relationships to the MCI population. In addition, this analysis incorporates CSF biomarkers known to characterize MCI subjects with AD pathology. The combination of total tau concentrations and the p-tau$_{181p}$: Aβ1–42 ratio predicts the categorical endpoint of conversion to AD with relatively good sensitivity and specificity [5, 10, 11, 15]. The current analysis focuses on continuous measures of disease progression such as ADAS-cog rather than the commonly reported categorical end points such as conversion or time to conversion. The emphasis of this analysis is on the mixing distribution for ADAS-cog change and that for baseline CSF biomarkers in MCI subjects. The objective is to assess the degree of correlation between rate of disease progression as measured by a continuous scale such as ADAS-cog and baseline CSF biomarker status. This information could be utilized to enrich clinical trials and may thus enable successful clinical trials in MCI subjects. The availability of richly sampled long term naturalistic MCI progression data from the ADNI public database (available at https://www.loni.ucla.edu/ADNI) allows assessment of (a) variabil-

ity in this disease state, (b) potential covariates affecting MCI progression and (c) the ability of ADAS-cog to track disease progression during the MCI stage. We thus aimed at developing a non-linear mixed effects model with covariates, incorporating neuropsychological assessments and structural or chemical biomarkers to describe disease progression in ADNI MCI subjects.

Methods

Study details

Data used in the preparation of this article were obtained from ADNI database (http://www.loni.ucla.edu/ADNI); for up-to-date information, see http://www.adni-info.org. All ADNI subjects had clinical/neuropsychological assessments and 1.5T MRI measurements, while CSF measurements were performed in only 50% of subjects. MCI subjects were assessed at 0, 6, 12, 18, 24 and 36 months, while AD subjects were assessed at 0, 6, 12 and 24 months. ADNI allows public access to all accumulating data. The dataset available on November 9 2010 (http://www.loni.ucla.edu/ADNI) was utilized in the current analysis. This recent download of the database contains 1036 ADAS-cog measurements from 198 MCI subjects with baseline CSF data. 42.4% of these MCI subjects have converted to AD at the time of the data download. Other plasma biomarkers of Aβ pathology were not assessed in the current analysis. A recent report based on the ADNI data shows that plasma Aβ shows mild correlation with other biomarkers of Aβ pathology and is rather insensitive because health conditions other than AD are also associated with altered concentrations of plasma Aβ [16]. More importantly, plasma Aβ has limited value for disease classification and modest value as a prognostic factor for clinical progression [16] and is not considered further.

The database also contained 88 AD subjects with CSF data. The data from the AD subjects were used only for exploratory purposes to visualize differences between AD and MCI subjects. The data from AD subjects were not used in the current model building exercise. A description of the objective behind each stage of the modelling procedure described below is provided in Table S1.

Data analysis software

Data set preparation was performed using SAS® Version 9.1.3 (SAS Institute Inc., Cary, NC, USA). Data set exploration and visualization were performed using S Plus® 6.0 professional release 2 software (Insightful Corporation, Seattle, WA, USA). ADAS-cog and CSF biomarker data were modelled using extended least squares regression using NONMEM® VI in combination with the Intel FORTRAN 10 compiler [17].

Selection of the structural model for ADAS-cog data

The model-building exercise employed log-transformed data using the first-order conditional estimation method (FOCE) in NONMEM®. It is known that linear models are not sufficient for portraying cognitive decline in disease progression [18, 19]. The use of logistic curves to describe this non-linearity in cognitive decline is well accepted [13, 14, 19–22] and these functions offer the advantage that the model predictions do not fall outside the bounded scale of 0 to 70 for ADAS-cog. To justify the choice of the non-linear structural model, simpler linear and non-linear models were also tested (see Results).

A sequence of logistic models [23] was tested and these models allowed the progression rate to be the fastest around the inflection point of 42 points on the ADAS-cog scale [13, 14, 20, 24]. The generalized logistic model [23] that represents the rate of disease progression is as follows:

$$\frac{d\text{ADAS-cog}}{dt} = r \times \text{ADAS-cog}^{\alpha}\left[1-\left(\frac{\text{ADAS-cog}}{\text{ADAS-cog}_{max}}\right)^{\beta}\right]^{\gamma}; \quad (1)$$
$$\text{ADAS-cog}(0) = \text{ADAS-cog}_0$$

where, r is the rate parameter controlling disease progression, ADAS-cog_{max} is fixed at 70, ADAS-cog_0 is the baseline score at time zero and α, β and γ govern the shape of the progression curve and also control the inflection point. In the MCI database there are only four data points (4/1036: 0.4%) with ADAS-cog scores greater than 42 and therefore estimating any of the shape parameters maybe difficult with the current dataset (see Results). Three different logistic models were tested: (a) in the first model α and β were fixed at 1, while γ was fixed at 0.667, (b) in the second model α and γ were fixed at 1, while β was fixed at 2.39 and (c) in the third model β and γ were fixed at 1, while α was fixed at 1.52. Since the relationship between inflection point and the shape parameters can be derived [23], the fixed shape parameters in each model allow the inflection point to be 42, which is in line with the literature derived value [13, 14, 20, 24] (Table 1). The three models are non-nested and have the same number of parameters. The selection of the structural model was therefore guided by AIC criteria and the model with the lowest AIC value was considered the base structural model.

Inter-subject variability on baseline ADAS-cog was evaluated using a log normal distribution because the parameter had to be constrained to a value greater than zero with its distribution skewed to the right. The apparent coefficient of variation for inter-individual variability in baseline ADAS-cog was computed as the square root of omega (ω). Inter-individual variability on the rate parameter r was evaluated using an additive-error model. Rate of progression can be either positive or negative (disease can worsen or improve over time) in MCI subjects. It is therefore important to use an additive-error model for

Table 1
Summary of structural models

Model description	Progression rate	Inflection point	Fixed parameter†	Number of θs	AIC value
Logistic 1	$\frac{dADAS\text{-}cog}{dt} = r \times ADAS\text{-}cog\left[1-\frac{ADAS\text{-}cog}{70}\right]^{\gamma}$	$\frac{70}{1+\gamma}$	$\gamma = 0.667$	2	−1126
Logistic 2	$\frac{dADAS\text{-}cog}{dt} = r \times ADAS\text{-}cog\left[1-\left(\frac{ADAS\text{-}cog}{70}\right)^{\beta}\right]$	$\left(\frac{70^{\beta}}{1+\beta}\right)^{\frac{1}{\beta}}$	$\beta = 2.39$	2	−1128
Logistic 3	$\frac{dADAS\text{-}cog}{dt} = r \times ADAS\text{-}cog^{\alpha}\left[1-\frac{ADAS\text{-}cog}{70}\right]$	$\frac{\alpha \times 70}{1+\alpha}$	$\alpha = 1.52$	2	−1129

†In all three models the fixed parameter corresponds to an inflection point at an ADAS-cog score of 42.

parameter r, so that both types of progression can be captured. The coefficient of variation for inter-individual variability on the r parameter was computed as 100% × ω/population estimate. Since ADAS-cog scores were log transformed, an additive error model was used to describe the residual variability. The scores are non-negative and were increasingly variable as the value of the scores increased. Both these characteristics are captured adequately using the log-transform both sides approach for the residual error [25, 26]. This approach involves logarithmic transformation of both the observed data and model predictions, which induces normality and allows variance stabilization [25, 26]. The magnitude of the residual variability parameter was expressed as a standard deviation.

Mixture model for ADAS-cog data

It was observed that the inter-individual variability estimates for the progression rate parameter r in the base structural model was quite high (>100% coefficient of variation). The high variability is also visible in the longitudinal ADAS-cog scores in MCI subjects (see Results). This led to the hypothesis that the MCI population consists of a mixture of two sub-populations and mixing of these non-homogenous populations led to high inter-individual variability. These two sub-populations could represent fast and slow progressers. Slow progressers were defined as those having a lower r parameter and lower baseline ADAS-cog (and *vice versa* for fast progressers). To test the possibility of two sub-populations, mixture modelling, as implemented in NONMEM® VI [27–29], was applied to the ADAS-cog data. To allow flexibility, residual variability was allowed to vary between the two sub-populations.

Mixture models for baseline CSF biomarker data

Two-component mixture models were also fitted separately for each of the baseline CSF biomarker data (CSF Aβ1–42, tau, p-tau$_{181P}$, and p-tau$_{181P}$: Aβ1–42 ratio) under the assumption that the data are sampled from 2 different normal distributions. Since there is a single baseline measurement per subject, only one level of random effects was implemented using an additive error model. Both tau markers had right skewed distributions and therefore CSF tau, p-tau$_{181P}$, and p-tau$_{181P}$ A : β1–42 ratio were log transformed before analysis to satisfy the normality assumption. The thresholds for p-tau$_{181p}$, Aβ1–42 and p-tau$_{181p}$: Aβ1–42 were based on the densities of their bimodal distribution. The threshold is taken as the lowest point in the trough between the two peaks where the density curves of the two distributions for the mixture population meet (see Results).

Computation of % correct classification statistics

It was conjectured that MCI subjects with non-pathologic CSF could be the slow progressers, while subjects with pathologic CSF could be the fast progressers. For CSF Aβ1–42, subjects below the critical threshold (identified by the mixture model above) were considered having pathologic CSF. In contrast, for CSF p-tau$_{181P}$ and p-tau$_{181P}$: Aβ1–42 ratio, subjects above the critical threshold from the respective mixture models were considered to have pathologic CSF. To assess whether there could be a correlation between ADAS-cog progression and CSF status, % correct classification (%CC) [27] statistics were computed between each subject's *post hoc* estimate of sub-population assignment from the ADAS-cog mixture model and the CSF sub-population category based on the CSF biomarker threshold. The %CC was computed for each CSF biomarker, where CC is either pathologic CSF corresponding to fast progresser status or non-pathologic CSF corresponding to slow progresser status.

CSF biomarkers as covariates in the ADAS-cog base structural model

The %CC was high for CSF Aβ1–42, p-tau$_{181P}$, and p-tau$_{181P}$: Aβ1–42 ratio and therefore an assessment was made whether these could serve as categorical covariates in the ADAS-cog base structural model. The optimal threshold for dichotomizing these biomarkers into categorical covariates was fixed based on the mixture model for these biomarkers described earlier. Three separate ADAS-cog models were fitted, one with CSF Aβ1–42, one with p-tau$_{181P}$ and another with p-tau$_{181P}$: Aβ1–42 ratio,

and in all models the biomarkers were formulated as categorical covariates to affect both baseline ADAS-cog and r. These models had the same number of parameters and selection of the more optimal biomarker as a covariate was based on the AIC criteria. For completeness, once the optimal biomarker was selected, it was also tested as a continuous covariate on baseline ADAS-cog and r using (a) linear function, (b) power function and (c) log linear function and the choice of the functional form of the covariate was also based on AIC. The categorical covariate formalism also offers two other advantages that were also tested: (a) the assumption can be tested whether the slow progressers are non-progressers and (b) the assumption can be tested whether the residual variability between the two sub-populations is sufficiently different The model chosen after incorporation of CSF biomarkers in the ADAS-cog model will be referred to as the base reference model.

Assessment of applicability of AD model covariates to the MCI population

The development of the base reference model led to the observation that there are only 129 progressers in the current dataset. A covariate search on such a small database could cause identification of incorrect covariate relationships due to random noise. Moreover, such an analysis, which could be associated with low power, may identify spurious and/or exaggerated covariate relationships [30]. Therefore, further covariate search was guided by prior knowledge related to this disease area. Previous analysis of covariate relationships in the ADNI population has suggested that baseline ADAS-cog is affected by baseline hippocampal volume, baseline ventricular volume and years since dementia onset at baseline [13, 14]. Furthermore, the r parameter is associated with baseline age, APOE4, baseline cholesterol and baseline Trail B test [12, 13, 31]. Since the MCI progressers identified in the current analysis have AD pathology (high p-tau$_{181P}$: Aβ1–42 ratio), the relevance of these previously known AD covariates was also tested in the MCI population (except years since dementia onset, which is not relevant to MCI). NONMEM® VI was used to optimize and finalize the covariate model In the model, continuous covariates were modelled using a power function after normalization by the typical reference value (population median), while categorical covariates were introduced as fractional shifts [32]. All of the influential covariates from the previous AD analysis [13, 14] were added to the base reference model using the appropriate functional form [32]. Covariates introduced into the full model were then tested using backward elimination, a procedure described by Wahlby *et al*. [33], and the objective of this analysis was to develop the most parsimonious covariate disease progression model in MCI.

Finally, to assess the precision and stability of the final model, the parameter estimates were subjected to internal model evaluation [13, 14]. The evaluation consisted of a non-parametric bootstrap and a visual predictive check [13, 14, 34, 35]. Bootstrap analysis was performed using the package Perl Speaks NONMEM®, version PsN-3.1.0 [34].

Results

Subject characteristics

The characteristics of the ADNI MCI subjects with CSF information are shown in Table 2. Petersen *et al*. have recently reported the demographic and biomarker characteristics of all the 398 MCI subjects recruited in the ADNI trial [36]. The characteristics of the 198 MCI subjects with CSF information in the current analysis (Table 2) are almost identical to the statistics for the full set of 398 MCI subjects (similar distribution for age, APOE, gender, educational status and cognitive tests). This indicates that the subset of MCI subjects with CSF information represents a representative sample of the larger population. This subset of MCI subjects was between the ages of 55 to 89 years (mean ± standard deviation [SD] 75 ± 8). The subjects had, on an average 16 years (±3 SD) of education. Ninety-eight subjects (49.5%) had a family history of dementia with at least one parent having the disease. There was an apparent pattern for maternal transmission of the disease since 77 of the 98 subjects with a family history had mothers with dementia which is consistent with earlier reports in the AD literature [13]. 54% of MCI subjects were APOE ε4 carriers, where 43% had one ε4 allele and 11% had two ε4 alleles. MCI subjects also had relatively high serum cholesterol, with the mean cholesterol concentration being 198 mg dl^{-1} (± 43 SD), which is close to the high cholesterol cut-off of ≥200 mg dl^{-1}.

Choice of structural model

The results from the logistic structural model selection process are shown in Table 1. AIC values for the various structural models indicate that logistic model 3 with α shape parameter was the most suitable (i.e. lowest value among the three models tested). This model form has also been reported to describe AD disease progression quite well [13, 14]. To understand the behaviour of these structural models, the progression rate was plotted as a function of the current ADAS-cog score using the parameter estimates from each model. The results are presented in Figure S1, which indicate that the three separate exponents (α, β and γ) control both the inflection point and the initial shape of the curvature characterizing the relationship between progression rate and ADAS-cog. The model with α shape parameter had greater flexibility at low ADAS-cog scores, which is particularly relevant to the MCI population (Figure S1). It is therefore reassuring that this function was identified here and in previous work [13, 14] as a suitable structural model for describing ADAS-cog progression.

A linear model for disease progression was also tested and it resulted in an AIC value of −1094, which signifies

Table 2

Summary statistics for ADNI MCI subjects with CSF data

Variable name (abbreviation), units	All subjects (n = 198)	Mean (± SD) or n (%) Subjects with pathologic CSF* (n = 129)	Subjects without pathologic CSF† (n = 69)
Baseline MRI volumetric measures			
Ventricular volume (ml)	44.6 ± 24	42.3 ± 21	48.8 ± 28
Hippocampal volume‡ (mm^3)	3146 ± 528	3045 ± 468	3334 ± 583
Baseline chemical biomarkers			
Serum cholesterol, mg dl^{-1}	198 ± 43	202 ± 45	192 ± 39
Subjects with high cholesterol, ≥ 200 mg dl^{-1}	87 (44%)	60 (47%)	27 (39%)
CSF Aβ1–42	164 ± 55	134 ± 30	218 ± 49
CSF tau	103 ± 60	125 ± 63	62 ± 22
CSF p-tau_{181P}	35 ± 18	44 ± 16	19 ± 5
Log CSF p-tau_{181P} : Aβ1–42 ratio	–1.6 ± 0.7	–1.14 ± 0.4	–2.46 ± 0.4
Demographic and genetic factors			
Baseline age (AGE), years	75 ± 8	74 ± 7	75 ± 8
Apolipoprotein E genotype status (APOE4)			
0 allele	92 (46%)	40 (31%)	52 (75%)
1 allele	85 (43%)	69 (53%)	16 (23%)
2 alleles	21 (11%)	20 (16%)	1 (1.4%)
Family history of dementia (FHD)			
None	100 (51%)	60 (47%)	40 (58%)
Father	21 (11%)	12 (9.3%)	9 (13%)
Mother	65 (33%)	48 (37%)	17 (25%)
Both	12 (6.1%)	9 (7.0%)	3 (4.3%)
Gender (SEX)			
Male	132 (67%)	79 (61%)	53 (77%)
Female	66 (33%)	50 (39%)	16 (23%)
Years of education (EDU) at baseline	16 ± 3	16 ± 3	16 ± 3
Baseline cognitive tests			
ADAS-cog	11.7 ± 5	12.7 ± 5	9.9 ± 4
Mini-mental state exam (MMSE)	26.9 ± 2	26.8 ± 2	27.1 ± 2
Trail making test; part B, s	133 ± 73	140 ± 74	121 ± 69
Longitudinal ADAS-cog scores	Mean ± SD (n)	Mean ± SD (n)	Mean ± SD (n)
Baseline	11.7 ± 5 (n = 198)	12.7 ± 5 (n = 129)	9.9 ± 4 (n = 69)
6 months	12.5 ± 5 (n = 190)	13.7 ± 5 (n = 125)	10.1 ± 5 (n = 65)
1 year	12.6 ± 6 (n = 184)	14.2 ± 6 (n = 121)	9.6 ± 4 (n = 63)
1.5 years	13.5 ± 7 (n = 169)	15.6 ± 7 (n = 111)	9.6 ± 5 (n = 58)
2 years	14.0 ± 7 (n = 158)	16.3 ± 7 (n = 106)	9.3 ± 5 (n = 52)
3 years	15.2 ± 9 (n = 118)	17.8 ± 9 (n = 76)	10.5 ± 6 (n = 42)

*Subjects with pathologic CSF at baseline: log CSF p-tau_{181P} : Aβ1–42 ratio > –1.86. †Subjects without pathologic CSF at baseline: log CSF p-tau_{181P} : Aβ1–42 ratio ≤ –1.86. ‡Average of left and right hippocampal volume.

poorer model fit compared with the logistic models (Table 1). A simplified logistic function was tested next, which does not have a shape parameter (i.e. characterized by an inflection point at half-maximal score of 35) and this model produced an AIC of –1125. It should be noted that all the logistic models that had a shape factor gave better AIC values (Table 1) than the simplified logistic model. This behaviour agrees with the published literature [13, 14, 20, 24] that the inflection point for ADAS-cog is close to 42 and not at the mid-point of the ADAS-cog scale. Finally, the logistic model with the α shape parameter that had the lowest AIC value was also rerun where α was estimated instead of being fixed. The model ran successfully and gave an estimate of α of 1.48 (inflection point = 41.8); which is very close to the fixed value of α = 1.52 based on prior knowledge. However, estimating α led to poorer parameter precision and therefore α was kept fixed at 1.52 based on extensive knowledge [13, 14, 18–22, 24] about the temporal nature of cognitive decline to ensure model stability. In summary, this exercise of testing various structural models confirmed the utility of the AD structural model for the MCI population, which is not surprising since 42% of the current MCI population converts to AD during the course of the study. The logistic structural model with the α shape parameter was thus taken forward for assessment of mixture populations and covariate analysis and is referred to as the base model.

Mixture model for ADAS-cog

Results from the base model indicated that the between subject variability for the progression rate parameter was 113% coefficient of variation. This led to the formulation of a mixture model for MCI ADAS-cog data. The parameter estimates of the mixture model are shown in Table S2,

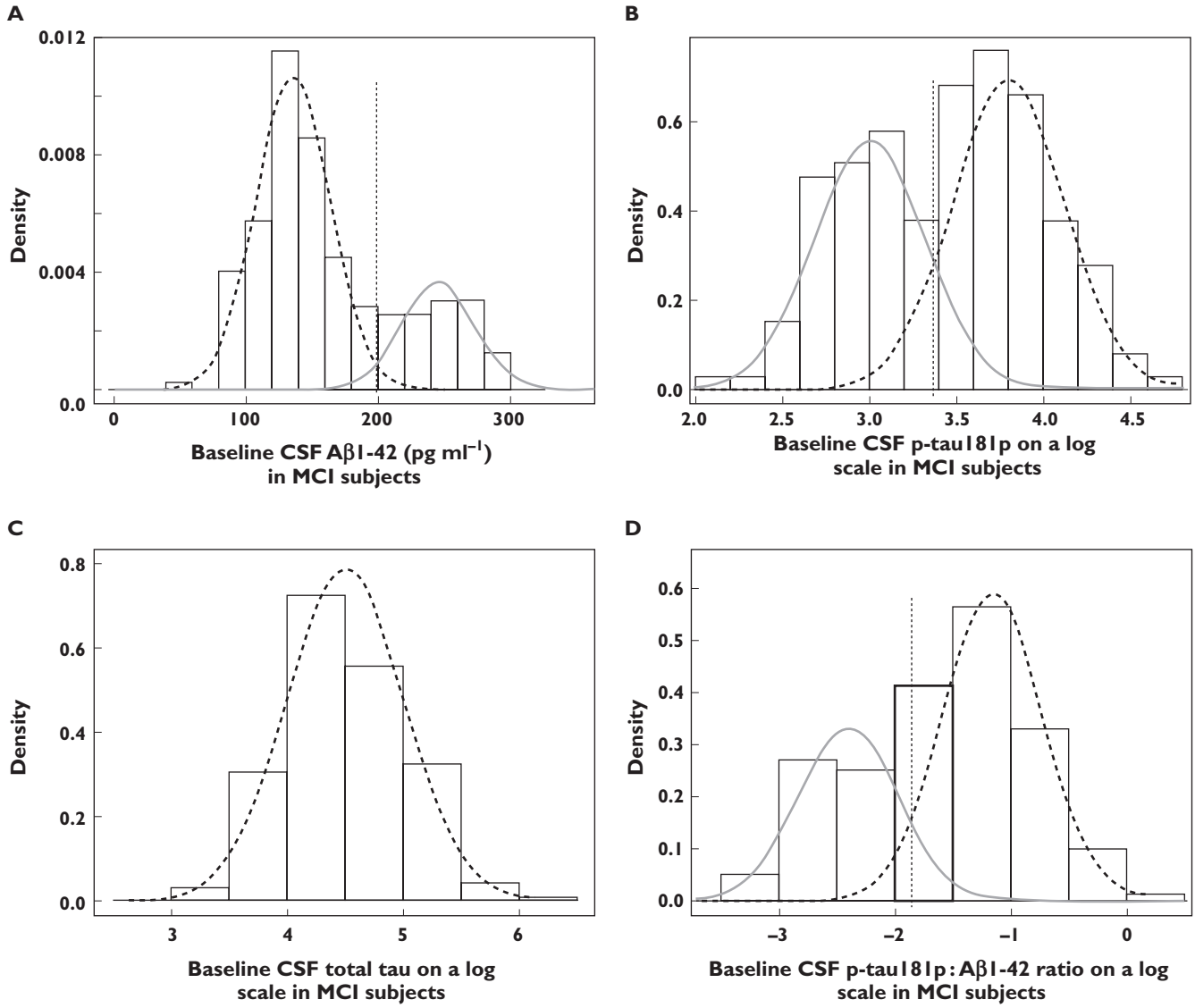

Figure 1

Distribution of CSF biomarkers in MCI subjects. The solid and dashed lines (1A, 1B and 1D) represent the density of the sub-populations based on the parameters of the CSF mixture models while the dotted vertical lines are the cut-off thresholds separating the two sub-populations. (A, B, D) - - -, With disease pathology; ——, Without disease pathology

which indicate that slow progressers have both a lower progression rate and a lower baseline score. The progression rate is even slower after accounting for the lower ADAS-cog scores observed in the MCI population (the model uses the logistic structural form). The mixing fraction for progressers was 70%, indicating that 30% of the subjects could be progressing slowly in the MCI population. To understand the biological basis behind this heterogeneity in the MCI population, CSF biomarkers were assessed for bimodality. The bimodality in progression rate could be associated with dichotomy in the distribution of CSF biomarkers. Therefore, mixture models for CSF biomarkers were assessed next.

Mixture models for CSF biomarkers and %CC statistics

Out of the four CSF candidate markers, three depicted possible bimodality (Figure 1). CSF total tau exhibited unimodality and a right skewed distribution, which was log-transformed to approximate normality (Figure 1C). A mixture model could not be successfully fitted to the log-transformed CSF total tau distribution and it was therefore not considered further as a candidate marker. For the three remaining markers a mixture model was successfully fitted and the results of the analysis are presented in Table S3. A mixture of two normal distributions with nearly equal standard deviations is bimodal if their means differ by at least

twice the common standard deviation [37]. This expectation of bimodality is met for CSF Aβ1–42 (Figure 1A), CSF p-tau$_{181p}$ (Figure 1B) and CSF p-tau$_{181p}$: Aβ1–42 ratio (Figure 1D) based on the parameter estimates reported in Table S3. The ability to fit mixture models with distinct random effect parameters is dependent upon the nature of the underlying mixture (i.e. how close are the sub-population means and how much data are available per sub-population). Attempts to fit separate random effects for CSF sub-populations led to model instability which was reflected in higher imprecision for model parameters. Thus the two sub-populations for the CSF biomarkers were assumed to have the same variances (Table S3) as is commonly done in the implementation of mixture models in NONMEM® [28, 29]. Furthermore, fitting the model without subpopulations to the baseline CSF dataset for p-tau$_{181p}$, Aβ1–42 and p-tau$_{181p}$: Aβ1–42 ratio resulted in much worse fit (based on AIC and likelihood ratio test).

The mixing proportion for p-tau$_{181P}$, p-tau$_{181P}$: Aβ1–42 and Aβ1–42 were 55%, 64% and 75% respectively and these are close to the 70% mixing proportion for the ADAS-cog mixture model. The thresholds determined for p-tau$_{181P}$, p-tau$_{181P}$: Aβ1–42 and Aβ1–42 based on the densities of these bimodal distribution were 29 pg ml^{-1} (log scale 3.37), 0.156 (log ratio −1.86) and 198 pg ml^{-1} respectively and these thresholds are indicated in Figure 1. Based on these threshold values, the population was dichotomized and the %CC statistic was computed for each marker using the *post hoc* estimate of sub-population assignment from the ADAS-cog mixture model. The %CC for p-tau$_{181P}$, p-tau$_{181P}$: Aβ1–42, Aβ1–42 were 68%, 73% and 71% respectively. Since the %CC for p-tau$_{181P}$, p-tau$_{181P}$: Aβ1–42 and Aβ1–42 were relatively high (~70%), all three markers were pursued further as potential covariates in the ADAS-cog base model. Since p-tau$_{181P}$: Aβ1–42 ratio gave the highest %CC statistic, the contingency table between CSF status and progresser status from the mixture model is reported in Table S4.

CSF biomarkers as covariates for ADAS-cog disease progression

CSF Aβ1–42 was incorporated as a categorical covariate on both baseline ADAS-cog and r parameter, which produced an AIC value of −1163. The AIC value with the model parameterizing CSF p-tau$_{181P}$ as the covariate was −1167. Finally, the AIC value for the model with CSF p-tau$_{181P}$: Aβ1–42 ratio as a covariate on the same parameters was −1181. This suggested that the ratio of the two biomarkers may carry more information than a single biomarker alone and it was chosen as the CSF-related covariate in the ADAS-cog model. For completeness, the ratio of p-tau$_{181P}$: Aβ1–42 was also tested as a continuous covariate through a linear, log-linear or power relationship, which yielded AIC values of −1165, −1179 and −1173. CSF p-tau$_{181P}$: Aβ1–42 ratio thus produces the lowest AIC value when it is formulated as a categorical covariate. This suggests that these CSF end points (p-tau$_{181P}$ and Aβ1–42) may serve as a threshold between occult and measureable disease progression. It is noteworthy that simply adding these two parameters to the ADAS-cog base model, p-tau$_{181P}$ A : β1–42 affecting baseline ADAS-cog and r, reduced the minimum value of the objective function by 56 points which is highly significant ($P < 0.00001$). It was also noticed that the estimate of the r parameter in the slow progressers (log p-tau$_{181P}$: Aβ1–42 $\leq$ −1.86) was 0.005, which is very close to zero. Therefore, the assumption was tested whether these subjects represent non-progressers with a typical r parameter value of zero. This simplification led to an increase in the minimum value of the objective function by 0.4 points. Therefore, the model reduction by one parameter did not lead to a significant change in the fit. Thus, based on this analysis, fast progressers will be referred to as progressers, while slow progressers will be referred to as non-progressers. Progressers are defined as subjects with log p-tau$_{181P}$: Aβ1–42 > −1.86, while non-progressers are defined as subjects with log p-tau$_{181P}$: Aβ1–42 $\leq$ −1.86.

The non-progressers, because of their small sample size ($n = 69$), were also constrained to have their etas (η: deviation of an individual parameter from the population mean) sampled from the same ω distribution as that of the progressers. However, to allow greater flexibility the residual variability was allowed to vary between progressers and non-progressers. Addition of one extra residual error parameter led to an improvement of 18 objective function points. It was also noticed that the SD of the residual error for the non-progressers (0.30) was somewhat larger than that for the progressers (0.24). This is because the ADAS-cog score for the non-progressers fluctuates more widely around a relatively steady value. This model in which the non-progressers had a typical progression rate parameter of zero, possessed a lower baseline score and were allowed to have a different residual variability was considered the base reference model and was tested further for covariate model building.

Final covariate model and model verification

Further covariate model building proceeded via a full model/backward elimination procedure in NONMEM® VI. The procedure identified only two new covariates in the model, which were hippocampal volume and the Trail B test (Table 3). Plots of baseline ADAS-cog η for progressers and non-progressers *vs.* hippocampal volume showed that the baseline score was dependent on this volumetric marker for both these populations. Furthermore, since the non-progressers represent a smaller fraction of the whole population ($n = 69$) only a single hippocampal volume related parameter was fitted for baseline ADAS-cog in the entire MCI population. The η for the r parameter *vs.* Trail B test score in non-progressers did not show any trend and therefore this covariate influences only the progressers.

Table 3

Population parameters and the precision of the parameters using nonparametric bootstrap

Parameter*	Original dataset Estimate	90% CI		Bootstrap replicates (n = 1000) Median	90% CI	
$\theta_{ADAS\text{-}cog0}$	11.3	10.7	11.9	11.3	10.7	11.9
θ_{HVOL}	−0.863	−1.10	−0.629	−0.878	−1.11	−0.627
θ_{CSF}	0.827	0.747	0.907	0.827	0.756	0.908
θ_r	0.042	0.034	0.049	0.041	0.034	0.048
θ_{TRAB}	0.621	0.379	0.863	0.634	0.394	0.862
Inter-subject variability (% coefficient of variation)†						
ADAS-cog$_0$	32.2	28.5	35.9	31.8	28.0	35.4
r	69.4	48.0	90.8	69.4	48.3	92.4
Residual variability (SD)						
Population with pathologic CSF‡	0.237	0.207	0.267	0.233	0.208	0.269
Population without Pathologic CSF‡	0.300	0.269	0.331	0.298	0.269	0.332

*These equations describe the relationships between covariates and the typical value (TV) of the parameters in the final model:

$$TV\ ADAS\text{-}cog_0 = \theta_{ADAS\text{-}cog0} \times \theta_{CSF}^{csf} \times \left(\frac{HVOL}{3115}\right)^{\theta_{HVOL}}$$

$$TV\ r = \theta_r \times \left(\frac{TRAB}{109}\right)^{\theta_{TRAB}} \times CSF_{FLAG}$$

where; csf is a 0/1 exponent and CSF_{FLAG} is a 1/0 flag variable depending on sub-population with/without pathologic CSF respectively. HVOL, CSF and TRAB refer to hippocampal volume, cerebrospinal fluid and Trail B test respectively. †Between the base model and final covariate model the inter-subject variability SD estimates improved from 39.5% and 113% to 32.2% and 69.4% coefficient of variation respectively. ‡Population with pathologic CSF corresponds to log CSF p-tau$_{181P}$: Aβ1–42 ratio > −1.86; population without pathologic CSF corresponds to log CSF p-tau$_{181P}$: Aβ1–42 ratio ≤ −1.86.

Figure S2 shows the goodness of fit plots for the final model and Table 3 provides the estimates from the final population based disease progression model. The results of the non-parametric bootstrap analysis (Table 3) support the parameter estimates of the final model. The parameter estimates are similar to the median value obtained from the bootstrap technique and are contained within the 90% confidence interval. The observed scores, the visual predictive check, and median model prediction *vs.* time are displayed in Figure 2. These results confirm that the model is able to describe the ADAS-cog temporal profiles in MCI subjects since the majority of the observations fall within the 90% prediction intervals (Figure 2).

Discussion

Characteristics of MCI population identified based on model based analysis

Three key characteristics of the MCI population emerge: (a) the MCI population potentially represents a mixture of two sub-populations, (b) among the MCI progressers, some of the subjects progress at a relatively slower rate likely due to additional factors such as preserved executive function and (c) among the non-progressers 32 subjects (16% of the MCI population) had a value of the r parameter that was negative, which indicates that some non-progressers may have the ability to revert back to normal status. This type of variability in the clinical course of MCI subjects has been described previously [2].

Rationale for testing CSF biomarkers as covariates

In the previous AD analysis [13, 14], the CSF data were not used since they were available in only 88 subjects. However, 198 MCI subjects had CSF information, which represents a reasonable size sample for investigating CSF biomarkers as covariates. At the current time, diagnosis of AD requires presence of dementia. However, there has been speculation that individuals who are bound to develop AD can be identified earlier using CSF biomarkers [38]. Aβ and p-tau$_{181P}$ are an integral part of disease pathology and it is interesting that progression on a clinical scale (ADAS-cog) is mirrored in the ratio of log CSF p-tau$_{181P}$: Aβ1–42. The critical threshold identified for this ratio in the current analysis is −1.86 (untransformed scale 0.156). MCI subjects below this critical threshold do not appear to exhibit disease progression (Figure 2A). This probably indicates that these subjects either do not have the disease pathology or the pathologic cascade has not started yet.

Role of APOE and cholesterol

In previous models of AD progression both APOE ε4 and serum cholesterol have been identified as covariates that predicted faster disease progression [13, 14, 31]. Aβ, APOE and cholesterol are linked with one another [39–41] since the APOE ε4 allele is linked with disturbances in Aβ and cholesterol metabolism. In the current analysis, APOE ε4 and serum cholesterol were not identified as statistically significant covariates. Instead, Aβ1–42 and p-tau$_{181P}$ are covariates in the model. If the entire MCI population is

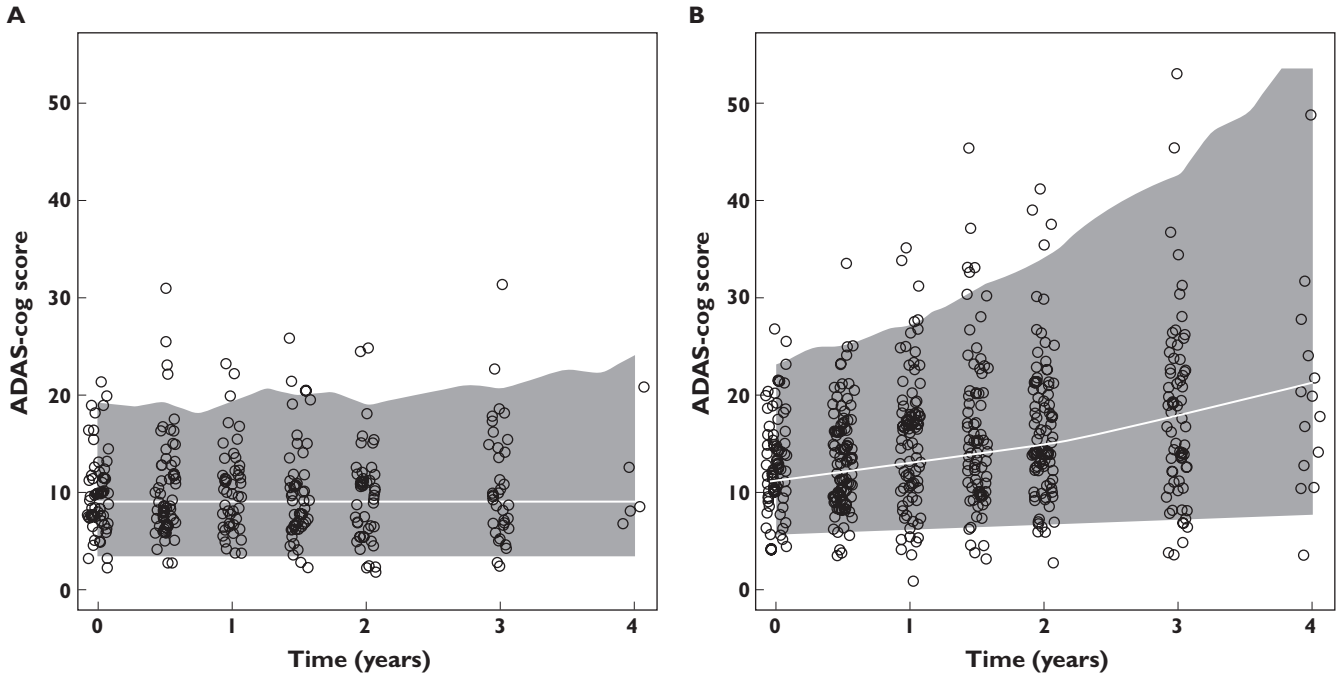

Figure 2

Results of the stratified visual predictive check; *x*-axis are jittered for clarity. Open symbols are observed data while lines and shaded areas represent the median and 90% prediction intervals. (A) Non-progressers without pathologic CSF [log CSF p-tau$_{181P}$: Aβ1–42 ratio ≤ −1.86] and (B) progressers with pathologic CSF [log CSF p-tau$_{181P}$: A β1–42 ratio > −1.86]

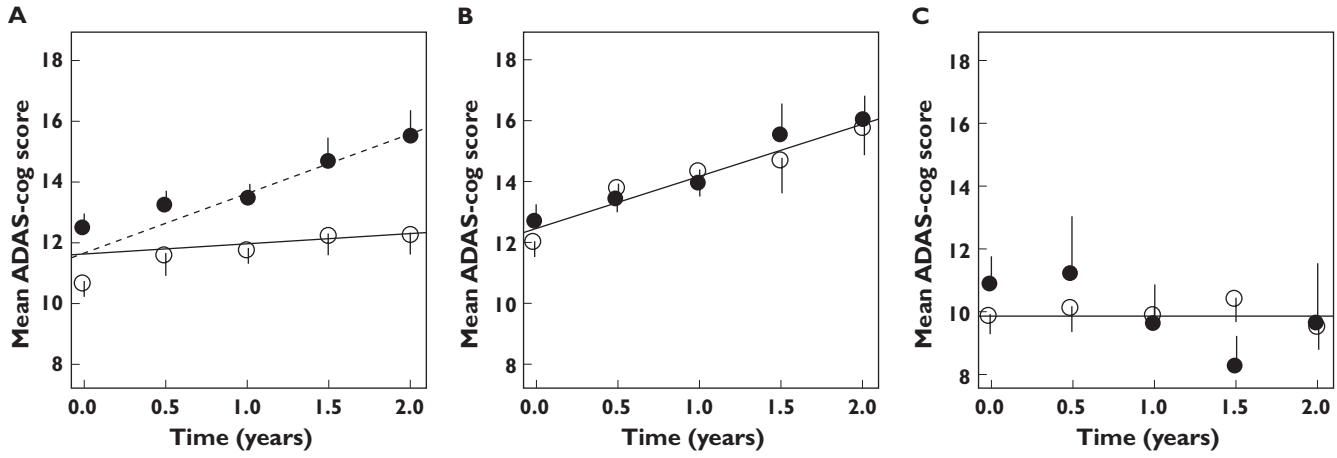

Figure 3

Influence of APOE may no longer be apparent once the data are dichotomized by CSF status. (A) entire MCI population, (B) progressers with pathologic CSF [log CSF p-tau$_{181P}$: Aβ1–42 ratio > −1.86] and (C) non-progressers without pathologic CSF [log CSF p-tau$_{181P}$: Aβ1–42 ratio ≤ −1.86]. APOE ε4 was dichotomized into carrier (one or two alleles) and non-carrier status. Error bars represent standard error (SE) and lines are simple linear regression through the data to allow visualization of trends. ○ APOE4 non-carrier; ● APOE4 carrier

stratified by either APOE or cholesterol status (Figures 3A and 4A respectively) there is an evident trend that these factors affect progression rate. However, if the population is first dichotomized by p-tau$_{181P}$: Aβ1–42 CSF status and the influence of APOE and cholesterol are assessed, then the trend disappears (Figures 3B, 3C and 4B, 4C respectively). Furthermore, 84% (89/106) of APOE ε4 carriers have the pathologic CSF ratio. Similarly 69% (60/87) of MCI subjects with high cholesterol have pathologic CSF ratio. Thus APOE ε4, high cholesterol and high p-tau$_{181P}$: Aβ1–42 ratio are likely correlated with one another. This probably also explains why high cholesterol and APOE ε4 were signifi-

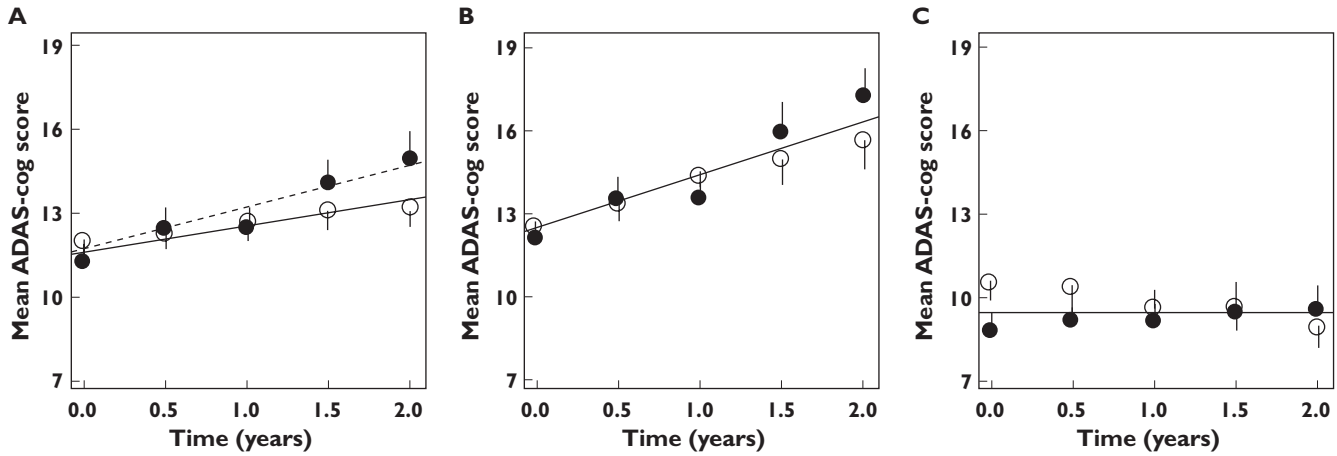

Figure 4

Influence of cholesterol is no longer apparent once the data are dichotomized by CSF status. (A) entire MCI population, (B) Progressers with pathologic CSF [log CSF p-tau$_{181P}$: Aβ1–42 ratio > −1.86] and (C) non-progressers without pathologic CSF [log CSF p-tau$_{181P}$: A^{-1}β1–42 ratio ≤ −1.86]. Total serum cholesterol was dichotomized into high cholesterol (≥200 mg dl^{-1}) and normal cholesterol (<200 mg dl^{-1}). Error bars represent standard error (SE) and lines are simple linear regression through the data to allow visualization of trends. (A, B, C) ○ normal cholesterol; ● high cholesterol

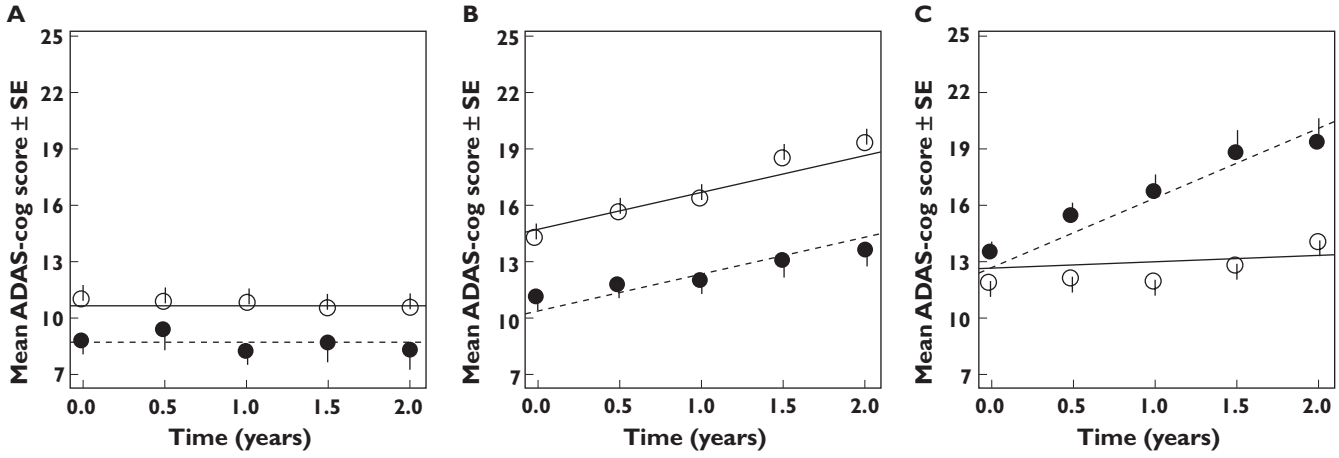

Figure 5

(A) Influence of hippocampal volume on baseline disease score for non-progressers, (B) impact of hippocampal volume on baseline score for progressers and (C) disease progression rate affected by Trail B test in progressers. Covariates were dichotomized to create roughly equal groups (> Median and ≤ Median) in each panel. Median hippocampal volume in progressers and non-progressers were 3045 and 3334 mm^3 respectively. Median Trail B test in progressers was 109 s. Error bars represent standard error (SE) and lines are simple linear regression through the data to allow visualization of trends. (A, B) ○ low hippocampal volume; ● high hippocampal volume. (C) ○ lower Trail B test time; ● higher Trail B test time

cant in the AD analysis where CSF biomarkers were not tested [13, 14, 31]. The p-tau$_{181P}$: Aβ1–42 ratio was highly significant in the MCI disease progression model and maybe a more useful covariate than APOE ε4 and cholesterol.

Other comparisons between MCI and AD progression models

Hippocampal volume and the Trail B test have been previously identified as influential covariates in AD [13, 14], which are equally significant in the current MCI analysis. To allow visualization of important covariate effects, some simple diagnostics were created (Figures 5, 6, and Figure S3). For these plots the important covariates were dichotomized (> Median and ≤ Median) to create roughly equal groups and the mean ADAS-cog was plotted as a function of this newly created categorical variable. Hippocampal volume was associated with baseline scores for both progressers and non-progressers, (Figure 5A and 5B). This finding for hippocampal volume is consistent with the literature where cognitive decline was associated with hippocampal atrophy [42]. Additionally, a longer completion

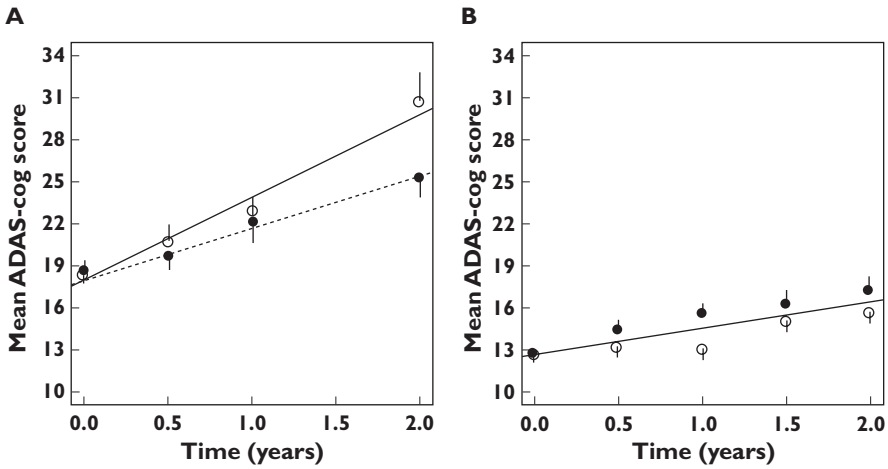

Figure 6

Differential effect of age on (A) AD subjects *vs*. (B) MCI progressers. For dichotomization the median age for AD subjects and MCI progressers were 76 and 74 years respectively. Error bars represent standard error (SE) and lines are simple linear regression through the data to allow visualization of trends. (A, B) ○ lower age group; ● higher age group

time on the Trail B test was associated with faster progression for subjects with pathologic CSF (Figure 5C), which indicates that patients with poor executive function progress rapidly.

Two additional covariates (ventricular volume and age) that were identified previously in AD [13, 14] were not statistically significant in the MCI analysis. The inability to identify ventricular volume in the current analysis is probably related to the narrow range of the baseline data in MCI. There is an apparent trend for the influence of ventricular volume on baseline ADAS-cog (Figure S3) but this trend does not reach statistical significance. As far as the influence of age is concerned, it does appear that there is a differential effect of this covariate on AD *vs*. MCI subjects (Figure 6). It seems that if the onset of AD dementia occurs at an early age then the form of the disease is rather aggressive and progression is quite rapid (Figure 6A). However, in the MCI population, the onset of dementia has not yet occurred and age does not appear to influence disease progression substantially (Figure 6b).

Summary of findings: application of CSF biomarkers for trial enrichment

The CSF findings from this analysis match with the ADNI information about the number of MCI subjects who have either converted ($n = 84$) or not converted ($n = 114$) to AD. The information about converters and non-converters from ADNI, as a function of CSF biomarker status, is depicted in Figure 7. The results indicate that the CSF information, at the individual level, has good negative predictive value i.e. 58 out of the 69 (84%) subjects with log p-tau$_{181P}$: Aβ1–42 ratio ≤ -1.86 have still not converted to AD. Moreover, it is also reassuring to see that 87% (73/84) of the converters have high log p-tau$_{181P}$: Aβ1–42 ratio (> -1.86). In contrast, 56 out of the 129 subjects (43%) with log p-tau$_{181P}$: Aβ1–42 ratio > -1.86 have still not converted to AD. These subjects likely will either (a) eventually develop AD as the 2–3 follow-up period in the current database may not be long enough or (b) it is also possible that these subjects have other protective factors (e.g. preserved executive function) that temporarily slow down their progression rate. Thus these CSF biomarkers may not precisely predict clinical conversion to AD. They can, however, be quite useful in excluding those subjects who have a low likelihood of exhibiting disease progression within a 2–3 year time frame of a clinical trial. Since non-progressing subjects may cause noise in an MCI clinical trial (higher residual error), it may be prudent to exclude them. The utility of CSF biomarkers as a trial enrichment tool has recently received regulatory attention in a qualification opinion issued by the European Medicines Agency [43]. Furthermore, there is at least one pharmaceutical company that is using this technique for population enrichment [44] and there are two distinct reasons for excluding these patients in a prodromal AD study: (i) they are likely to remain stable on both placebo and active arms and (ii) these subjects likely do not have Aβ and tau abnormalities and may not benefit from a therapy directed towards plaque and tangle pathology.

Two recent publications report results that are quite compatible with the current analysis. First, Buchhave *et al*. report a clinical study from Sweden with median follow-up time of 9.2 years in 137 MCI patients [45]. In this study baseline p-tau$_{181p}$: Aβ1–42 ratio again exhibited bimodality and 90% of the patients with pathologic CSF biomarker levels (high p-tau$_{181p}$ and low Aβ1–42) developed AD in 9–10 years. Secondly, Snider *et al*. report another smaller study with 49 MCI subjects with longitudinal profile for clinical dementia rating-sum of boxes which is another cognitive and functional end point [46]. Their results also

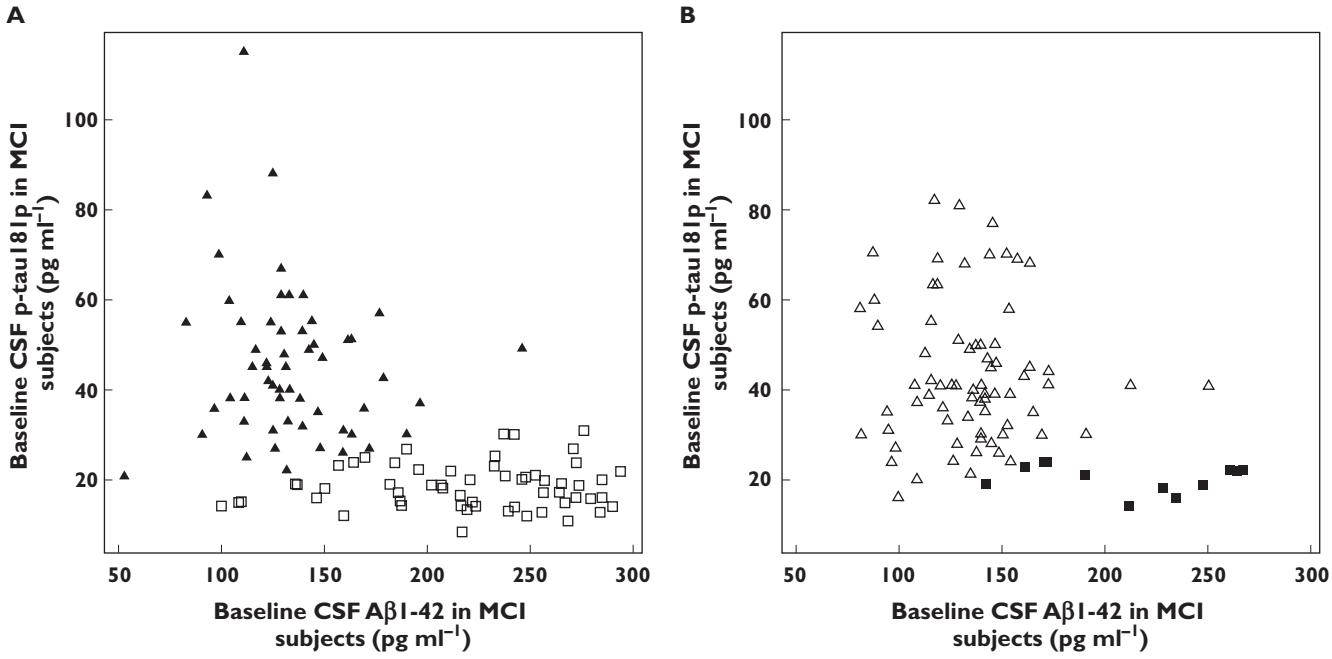

Figure 7

Relationship between CSF p-tau$_{181P}$ and Aβ1–42 for MCI subjects that have (A) not converted to AD and (B) converted to AD. In both panels triangles represent subjects with log CSF p-tau$_{181P}$: Aβ1–42 ratio > –1.86, while squares represent subjects with log CSF p-tau$_{181P}$: Aβ1–42 ratio ≤ –1.86. In both panels open symbols refer to correct assignment i.e. low ratio subjects who do not convert and high ratio subjects who convert to AD. In contrast, filled symbols represent incorrect assignment i.e. high ratio subjects who have not converted and low ratio subjects who have converted to AD. (A) ▲ log CSF p-tau$_{181p}$: Aβ1-42 ratio > –1.86; □ log CSF p-tau$_{181p}$: Aβ1-42 ratio ≤–1.86. (B) △ log CSF p-tau$_{181p}$: Aβ1-42 ratio > –1.86; ■ log CSF p-tau$_{181p}$: Aβ1-42 ratio ≤–1.86

show that high p-tau$_{181p}$ and low Aβ1–42 quantitatively predicts rapid progression for cognitive decline. Thus the current analysis and previous results qualify the utility of CSF biomarkers for being predictive of the rate of cognitive decline on continuous scales rather than just the dichotomous outcome of conversion to AD.

It should be pointed out that MCI clinical trials have historically used categorical or time to event outcomes as their primary analysis [47]. Recently Donohue *et al*. have suggested that continuous assessment of disease severity may be more efficient because mixed-effects models use all available data, which make them more robust [47]. Donohue *et al*. have also shown that trials with continuous outcomes have greater power on average than those with a dichotomous outcome [47]. Thus, the mixed effects disease progression model presented in the current analysis could also find utility in analyzing data from pivotal efficacy trials in MCI.

In summary, this work provides an integrated model-based analysis of disease progression in MCI subjects. This model allows identification of sub-populations suitable for trial enrichment and could represent a useful tool for efficient trial design through clinical trial simulations. In particular, CSF biomarkers can be useful for excluding those MCI subjects who have a low likelihood of exhibiting disease progression on both continuous and categorical end points. Furthermore, continuous end points may be more suitable than categorical endpoints since they have the potential to increase the statistical power of clinical trials.

One of the obstacles for implementing the trial enrichment approach is the variation in biomarker measurements observed between studies and laboratories. Even though these biomarker distributions show bimodality at baseline in MCI studies [5, 45] the absolute values for these biomarkers can be quite different. This variation is probably the result of differences in CSF sample handling techniques, analytical procedures and analytical kits/reagents. Standardization of these procedures may reduce the variation and increase the utility of these CSF biomarkers. Currently, there are at least three quality control and standardization initiatives [48–50] underway that will likely help with harmonization of CSF biomarker measurements.

Competing interests

The authors of this manuscript are employees of Johnson & Johnson Pharmaceutical Research & Development (JnJPRD) and own JnJ stock.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1
Derived shapes of the progression rate curve as a function of the current ADAS-cog score. Three structural models with different shape parameters were tested and the results of the assessment are shown below

Figure S2
Goodness-of-fit plots for the final model. (A) observed *vs*. population and individual predictions. The solid line represents the line of identity, (B) population weighted residuals vs. time and population predictions and (C) individual residuals *vs*. individual predictions, and distribution of population weighted residuals. Ordinate value of zero is presented in all the residual plots (solid line). Dashed line represents the LOWESS smoother. On the bottom right panel, the solid line represents the normal density and the dashed line represents the kernel density of population weighted residuals

Figure S3
Influence of ventricular volume on disease progression: (A) non-progressers without pathologic CSF [log CSF p-tau$_{181P}$: Aβ1–42 ratio ≤ -1.86] and (B) progressers with pathologic CSF [log CSF /p-tau$_{181P}$: Aβ1–42 ratio > -1.86]. Ventricular volumes were dichotomized to create roughly equal groups (> Median and ≤ Median) in the left and right panels where median ventricular volumes were 42.9 ml and 38.4 ml. Error bars represent standard error (SE) and lines represent simple linear regression through the data to allow visualization of the trends

Table S1
Elucidation of the motivation behind each stage of the modelling procedure

Table S2
Mixture model parameters for ADAS-cog scores from ADNI MCI subjects with CSF data

Table S3
Parameters of the mixture models fitted to the baseline CSF biomarker data in the ADNI MCI subjects. The MCI population was dichotomized based on the thresholds and the %CC statistic is reported using the post-hoc estimate of the sub-population assignment from the ADAS-cog mixture model

Table S4
Contingency table between CSF status and progresser status from the mixture model