

Effects of non-steroidal anti-inflammatory drug treatments on cognitive decline vary by phase of pre-clinical Alzheimer disease: findings from the randomized controlled Alzheimer's Disease Anti-inflammatory Prevention Trial

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Objective: We examined the effects of non-steroidal anti-inflammatory drugs on cognitive decline as a function of phase of pre-clinical Alzheimer disease.

Methods: Given recent findings that cognitive decline accelerates as clinical diagnosis is approached, we used rate of decline as a proxy for phase of pre-clinical Alzheimer disease. We fit growth mixture models of Modified Mini-Mental State (3MS) Examination trajectories with data from 2388 participants in the Alzheimer's Disease Anti-inflammatory Prevention Trial and included class-specific effects of naproxen and celecoxib.

Results: We identified three classes: "no decline", "slow decline", and "fast decline", and examined the effects of celecoxib and naproxen on linear slope and rate of change by class. Inclusion of quadratic terms improved fit of the model ($-2 \log$ likelihood difference: 369.23; $p < 0.001$) but resulted in reversal of effects over time. Over 4 years, participants in the slow-decline class on placebo typically lost 6.6 3MS points, whereas those on naproxen lost 3.1 points (p -value for difference: 0.19). Participants in the fast-decline class on placebo typically lost 11.2 points, but those on celecoxib first declined and then gained points (p -value for difference from placebo: 0.04), whereas those on naproxen showed a typical decline of 24.9 points (p -value for difference from placebo: <0.0001).

Conclusions: Our results appeared statistically robust but provided some unexpected contrasts in effects of different treatments at different times. Naproxen may attenuate cognitive decline in slow decliners while accelerating decline in fast decliners. Celecoxib appeared to have similar effects at first but then attenuated change in fast decliners. Copyright © 2011 John Wiley & Sons, Ltd.

Key words: NSAIDs; Alzheimer; prevention; dementia; growth mixture models

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Introduction

The relationship between inflammation, non-steroidal anti-inflammatory drugs (NSAIDs), and Alzheimer disease (AD) is complex, as evidenced by conflicting

findings on the effects of NSAIDs on AD and AD risk. Given the public health impact of AD (Wimo *et al.*, 2006), and the widespread use of NSAIDs among the older individuals (Sloane Report, 2006), it is important to determine under what circumstances these medications

may be helpful or harmful. We will argue that the pre-clinical period of AD can be usefully divided into three phases and that these phases can be characterized by the rate at which individuals are declining. Further, we will argue that the role of inflammation in the pathogenesis of AD varies between these different pre-clinical phases. As such, the effects of NSAID exposure on cognitive decline would also be expected to vary by phase of pre-clinical AD.

Conflicting results from non-steroidal anti-inflammatory drug trials

Studies of the effects of NSAID exposure on AD and AD risk have yielded conflicting results. A number of observational studies suggest that NSAIDs are associated with reduced AD risk. A meta-analysis of seven non-prospective studies of AD and lifetime NSAID use yielded a combined odds ratio of 0.51 (95% confidence interval (CI): 0.40, 0.66), whereas three prospective studies yielded a combined relative risk of 0.42 (95% CI: 0.26, 0.66) (Szekely *et al.*, 2004). More recent studies, including the Cardiovascular Health Study (CHS) and a case-control analysis of 246,199 US veterans, also report reduced AD risk among NSAID users, although a 12-year cohort study of 1019 older Catholic clergy found no association (Arvanitakis *et al.*, 2008; Szekely *et al.*, 2008a; Vlad *et al.*, 2008).

By contrast, randomized placebo-controlled trials (RCT) of diclofenac, nimesulide, and naproxen failed to show an effect (Scharf *et al.*, 1999; Aisen *et al.*, 2002, 2003). One trial showed a positive effect of indomethacin, but a subsequent study failed to replicate that finding (Rogers *et al.*, 1993; de Jong *et al.*, 2008). Three trials of cyclooxygenase (COX) 2 specific inhibitors and one of aspirin also showed no benefit (Aisen *et al.*, 2003; Reines *et al.*, 2004; Soyninen *et al.*, 2007; AD2000 Collaborative Group *et al.*, 2008).

Non-steroidal anti-inflammatory drug prevention trials in mild cognitive impairment (MCI) and normals showed a potential increase in AD risk. A large RCT of rofecoxib MCI showed increased AD hazard (Thal *et al.*, 2005). Previously, in the Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT), we reported hazard ratio estimates of 1.99 (95% CI: 0.80, 4.97) and 2.35 (95% CI: 0.95, 5.77) for celecoxib and naproxen, respectively (ADAPT Research Group *et al.*, 2007). After exclusion of seven AD cases adjudicated to have been present at the time of randomization, these hazard ratio estimates increased to 4.11 (95% CI: 1.30, 13.0) and 3.57 (95% CI: 1.09, 11.7) (ADAPT Research Group *et al.*, 2007). Subsequent analyses of cognitive outcomes

demonstrated a significant difference in global summary score decline for naproxen ($\beta = -0.05$; 95% CI: $-0.09, -0.01$) as compared with placebo and significant Modified Mini-Mental State (3MS) Examination differences in decline for both celecoxib ($\beta = -0.32$; 95% CI: $-0.62, -0.02$) and naproxen ($\beta = -0.36$; 95% CI: $-0.68, -0.04$) as compared with placebo (ADAPT Research Group, 2008).

Phases of pre-clinical Alzheimer disease

Alzheimer disease develops over the course of decades, and the pre-clinical period can be usefully divided into three phases (Lyketsos *et al.*, 2008), which can be characterized by rate of cognitive decline. These phases are distinct and separate from previously described stages of AD, which follow clinical diagnosis. During the first phase, there is little or no cognitive decline. Supporting this period as a distinct phase are findings of stage A amyloid deposits and stage I/II neurofibrillary changes in the brains of individuals autopsied as young as 40 years, studies with ^{11}C -PET-PiB confirming the presence of amyloid in the brains of non-impaired older individuals, and magnetic resonance imaging studies showing brain atrophy in cognitively normal subjects (Braak and Braak, 1997; Aizenstein *et al.*, 2008; Fripp *et al.*, 2008; Fennema-Notestine *et al.*, 2009).

Several prospective studies of asymptomatic individuals with higher genetic risk have documented subtle memory declines, which predicted conversion to MCI and correlated with cerebral glucose metabolism (Reiman *et al.*, 1996; Casellie *et al.*, 2008). Additional long-term prospective studies have demonstrated differences on cognitive tests in individuals who ultimately developed dementia as early as 9 years prior to diagnosis and who experienced steeper declines 2–3 years prior to diagnosis (Small *et al.*, 2000; Chen *et al.*, 2001; Bäckman *et al.*, 2005; Small and Backman, 2007; Amieva *et al.*, 2008; Howieson *et al.*, 2008). These findings support further division of the pre-clinical period into slow (phase 2) and fast (phase 3) periods of cognitive decline. We would expect that a sample of older individuals without clinical diagnoses of dementia would contain individuals in each of these three pre-clinical phases (as well as some who were not destined to develop dementia). If followed, their cognitive trajectories could then be classified as belonging to one of the three cognitive decline phases. Such an analysis has been performed using growth mixture models (GMMs) and data from the Cambridge City over 75 Cohort Study, and the authors reported findings in support of three classes of trajectories based on patterns of mini-mental state exam scores over 21 years: one

class starting out unimpaired at baseline with a slow decline, a second starting out moderately impaired with sharper decline, and a third starting out moderately impaired but with an accelerating rate of decline (Terrera *et al.*, 2009).

Changing role of inflammation during the phases of pre-clinical Alzheimer disease

The pathogenesis and progression of AD are attended by a variety of inflammatory processes, but it is unclear whether these processes cause, are a byproduct of, or ameliorate the proliferation of amyloid plaques, neurofibrillary tangles, and neuronal loss (Akiyama *et al.*, 2000; Wyss-Coray, 2006). According to the amyloid cascade/neuroinflammation hypothesis, amyloid-beta ($A\beta$) activates microglial cells, which promote amyloid plaque formation (Akiyama *et al.*, 2000). This is supported by reports of $A\beta$ secretion by cultured microglia and by evidence that microglia are involved in the laying down of amyloid fibrils (Bauer *et al.*, 1991; Wisniewski and Wegiel, 1994; Bitting *et al.*, 1996). In autopsy studies of normal older individuals, microglia were associated with neuritic but not diffuse plaques (Mackenzie *et al.*, 1995; Sasaki *et al.*, 1997). Interleukin-1 over-expression by activated microglia has also been shown to cause tau phosphorylation and tangle development (Mrak and Griffin, 2005). Hence, inflammation in the form of microglial activation promotes AD neuropathy in the early phase(s) of pre-clinical AD.

Inflammatory processes can also be neuroprotective. Microglia also participate in plaque phagocytosis (Shaffer *et al.*, 1995; Paresce *et al.*, 1997). The cytokine interleukin-3 (IL-3) has been shown to inhibit $A\beta(1-42)$ -mediated neuronal death (Rojo *et al.*, 2008), and compared with normal older controls, those with mild AD have lower secretion of IL-3 by mononuclear cells (Huberman *et al.*, 1994). This suggests that inflammatory processes play a role in plaque formation, but some are also involved in plaque clearance and neuroprotection.

Non-steroidal anti-inflammatory drug effects on cognitive decline vary by timing of exposure

We hypothesize that the role of inflammation changes during the course of pre-clinical AD, such that it is harmful during the early phases (1 and 2), when plaques and tangles are being formed, but that once substantial numbers of plaques have formed (phase 3), inflammation is helpful and necessary for plaque clearance. Therefore, we would expect NSAID exposure during early phases to be protective but harmful during

the later phase. To date, there are no analyses stratifying individuals by pre-clinical phase. However, analyses stratified by age of NSAID exposure yield comparable results. In the CHS, reduced risk of AD in NSAID users was only significant in the younger group (Szekely *et al.*, 2008b). In the Cache County Study on Memory and Aging, individuals using NSAIDs before age 65 years had shallower cognitive decline compared with non-users, whereas decline for those who began after 65 years was steeper (Hayden *et al.*, 2007). In the Adult Changes in Thought study, hazard ratios for current heavy users was higher than those of remote users, suggesting a gradient of risk with timing of exposure (Breitner *et al.*, 2009). Finally, in the ADAPT study, when the study interval was divided into pre-defined early and late periods, risk of incident AD was increased early on in both the celecoxib and naproxen treatment groups for 54 individuals who had MCI at enrollment and who were likely in the third phase of pre-clinical AD (Breitner, 2009).

If the pre-clinical phase were an observed variable (e.g., measurable by some biomarker), our hypothesis could be tested using phase-stratified analyses. In the absence of such a biomarker, we will use GMMs to differentiate the pre-clinical phases as latent classes based on observed trajectories of cognitive decline. We will then test for latent class-specific effects of NSAID treatment. The objectives of these analyses are as follows: (i) to empirically confirm the existence of three classes of trajectories of cognitive decline (representing the three phases of pre-clinical AD) using longitudinal scores on the 3MS; (ii) to determine if NSAID treatment differentially affects rate of decline by phase of pre-clinical AD; and (iii) to validate the model by replicating the number of classes of trajectories, as well as the differential NSAID effects by class, on a second measure, the Dementia Severity Rating Scale (DSRS).

Methods

Study population and design

The ADAPT was a randomized, placebo-controlled, multisite primary prevention trial. Study methods have been previously reported in detail (ADAPT Research Group *et al.*, 2007). Participants were recruited through mailings to Medicare beneficiaries near the study's field sites (Baltimore, MD; Boston MA; Rochester, NY; Seattle, WA; Sun City, AZ; and Tampa, FL). Eligible participants were older than 70 years and had at least one first-degree relative with Alzheimer-like

dementia. This study was registered on ClinicalTrials.gov (#NCT00007189) and approved by local institutional review boards. Informed consent was obtained from each participant along with an informant. The 2528 participants received 200 mg of celecoxib bid (Celebrex, Pfizer, New York, NY), 220 mg of naproxen sodium bid (Aleve, Bayer HealthCare, Pittsburgh, PA), or matching placebo. Randomization was on a 1:1:1.5 basis, stratified by age and study site. At baseline and yearly, participants were evaluated with a cognitive assessment battery, including the 3MS (Teng and Chui, 1987) and the DSRS (Clark and Ewbanks, 1986). The 3MS is a widely used, 100-point global cognitive measure with higher scores indicative of better cognitive functioning. For parity with other ADAPT analyses, we used education-adjusted 3MS scores in all analyses (Elkins *et al.*, 2006; ADAPT Research Group, 2008). The DSRS is a multiple-choice questionnaire completed by a knowledgeable informant who rates impairment in 12 cognitive and functional domains. Scores range from 0 to 54; higher scores indicate greater impairment (Clark and Ewbanks, 1986). On 17 December 2004, treatment was suspended because of safety concerns, but yearly cognitive testing continued until September 2007 (ADAPT Research Group, 2008). Analyses were conducted as “intent-to-treat”, such that individuals stayed in their treatment groups even after treatment was halted.

Analytic methods

Growth mixture models allow for the grouping of individuals based on similarities among their trajectories on an outcome measured over time. They are based on an underlying assumption that each individual's scores over time are the result of their being a member of a latent (unobserved) class. For each latent class of trajectories, the average intercept, slope, and quadratic term are estimated. For each individual, probabilities of membership in each class are calculated based on how well their trajectory matches the mean trajectories of each of the classes, and these probabilities can be used to assign individuals. GMMs also allow for the inclusion of treatment effects on the slope and quadratic terms of each trajectory class, and these are analogous to interactions between treatment and time or time squared in standard longitudinal regression models. These models also allow for the inclusion of variables that predict class membership. For more details on GMMs, please see Appendix A.

After determining the appropriate number of latent trajectory classes, we added treatment effects for both

celecoxib and naproxen on the slope and quadratic terms. We compared a model in which the treatment effects on the trajectories were the same across all classes with a model in which they were allowed to be different by using a likelihood ratio test. We then added age and apolipoprotein E (*APOE*) genotype as predictors of class membership. We then compared numbers of individuals randomized to each treatment and mean time on treatment across latent classes. Ideally, the fitted model would be replicated in an independent sample. As it is unlikely that the ADAPT study will ever be replicated, instead, we replicated the class structure and treatment effects by using an additional measure, the DSRS. Programming details and model output are available from the first author upon request.

Results

Demographics and other sample information are reported elsewhere; treatment groups did not differ significantly on any baseline variables (ADAPT Research Group, 2008). Growth mixture models were run using data from 2388 individuals with complete covariate (age and *APOE*) information. Years on treatment did not differ by treatment assignment: placebo, 1.79 (1.05); celecoxib, 1.83 (1.02); naproxen, 1.81 (1.05). Based on fit statistics, bootstrapped likelihood ratio tests (BLRTs), and estimability, we chose a three-class model. Inclusion of the quadratic term improved fit ($-2 \log$ likelihood difference ($-2LLD$): 369.23; $p < 0.001$). A model allowing different treatment effects on trajectories between classes fit the data significantly better than a model in which treatment effects were constant across classes ($-2LLD$: 369.23; $p < 0.001$). The inclusion of *APOE* and age at baseline as predictors of class membership improved the fit further ($-2LLD$: 106.65, $p < 0.001$). Age was a significant predictor of baseline 3MS score. Table 1 shows estimates from the final model. The majority of individuals (86%) were in the “no-decline” class with high baselines and minimal declines. A smaller group (10%) was in a “slow-decline” class with lower baselines and shallow declines. The remaining 4% were in a “fast-decline” class with the lowest baselines and sharp declines. The three classes did not differ significantly by proportion of individuals on celecoxib and naproxen (class 1: 0.29, 0.29; class 2: 0.29, 0.28; class 3: 0.29, 0.29) or by mean years of treatment prior to discontinuation (class 1: 1.82(1.09); class 2: 1.79(1.04); class 3: 1.58(1.14)). As expected, *APOE* was significantly associated with increased log odds of

Table 1 3MS growth mixture model parameter estimates

	Class 1 (no decline)	Class 2 (slow decline)	Class 3 (fast decline)
Prevalence	0.86	0.10	0.04
Baseline 3MS score	94.73 (0.08) ^a	91.7 (0.27)	86.31 (0.28)
Rate of decline ^b	-0.32 (0.09)**	-0.84 (0.53)	5.63 (0.81)**
Change in rate of decline ^c	0.04 (0.02)*	-0.20 (0.07)*	-2.11 (0.11)**
Effect of age on baseline 3MS score	-0.13 (0.02)**		
Log odds of class membership			
Age		0.13 (0.03)**	0.07 (0.04)
APOE		1.12 (0.23)**	0.89(0.28)**
Effect of celecoxib on			
Rate of decline ^d	-0.20 (0.13)	-0.88 (0.68)	-2.87 (1.64)
Change in rate of decline ^e	0.06 (0.03)	-0.02 (0.11)	1.49 (0.25)**
Effect of naproxen on			
Rate of decline	0.05 (0.14)	-1.73 (0.75)*	-3.30 (0.97)**
Change in rate of decline	-0.02 (0.03)	0.65 (0.10)**	-0.03 (0.19)

3MS, Modified Mini-Mental State Examination; APOE, apolipoprotein E.

^aParameter estimate (standard error).

^bEffect of time.

^cEffect of time squared.

^dInteraction between treatment and time.

^eInteraction between treatment and time squared.

* $p < 0.05$; ** $p < 0.01$.

class membership in classes 2 and 3 relative to class 1. Higher age at baseline was significantly associated with membership in class 2.

Table 2 shows expected 3MS score changes over the first 4 years for each class. In class 1 (no decline), individuals in all three treatment groups were stable over 4 years. In class 2 (slow decline), the expected declines for placebo, naproxen, and celecoxib were 6.6, 3.1, and 10.5 points, respectively, but these differences were not statistically significant. In class 3 (fast decline), individuals on placebo were expected to lose 11.2 points, individuals on naproxen were expected to lose 24.9 points, but those on celecoxib were expected to gain 1.1 points. Both treatments were statistically significantly different from placebo. Figure 1 shows fitted trajectories (bold lines) for each

treatment group along with individual observed trajectories (thin lines) for all study participants.

We followed a similar model-fitting process with our validating outcome, DSRS. As with the 3MS, we chose a three-class model; a model with class-specific treatment effects fit better than a model with class-invariant treatment effects ($-2LLD = 34.77$, $p < 0.001$). Both APOE and age were significantly associated with membership in classes 1 and 2 ($-2LLD = 62.09$, $p < 0.001$). The direct effect of age on intercept was not statistically significant but was retained in the model to facilitate comparisons between the 3MS and DSRS models. Table 3 shows expected DSRS score changes over the first 4 years for each class. Figure 2 is analogous to Figure 1, but in order to facilitate comparisons, the vertical axes have been reversed.

Table 2 Predicted 3MS changes over 4 years per latent class and treatment group

	Class 1	Class 2	Class 3
Placebo	-0.59 (-1.33, 0.16) ^a	-6.62 (-10.62, -2.61)	-11.23 (-15.55, -6.91)
Naproxen	-0.74 (-1.3, -0.19)	-3.14 (-7.44, 1.17)	-24.9 (-28.49, -21.32)
Celecoxib	-0.52 (-1.01, -0.02)	-10.46 (-14.71, -6.21)	1.12 (-10.6, 12.83)
	$p = 0.27^b$	$p = 0.19$	$p < 0.001$
	$p = 0.34$	$p = 0.14$	$p = 0.04$

3MS, Modified Mini-Mental State Examination.

^aPredicted change in the 3MS score after 4 years for individuals in class 1 who were treated with placebo (95% CI).

^b p -value for the within-class comparison of predicted 4-year change in the 3MS score on naproxen versus placebo.

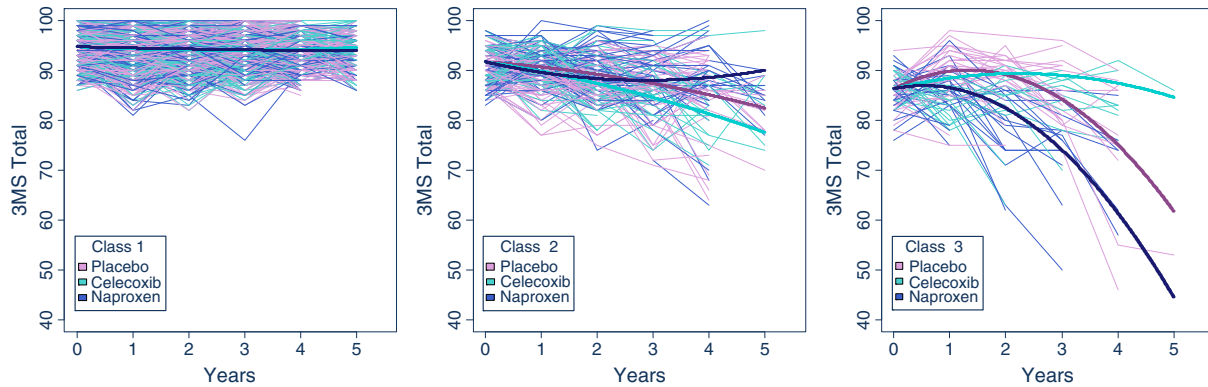


Figure 1 Predicted and observed 3MS trajectories per latent class and treatment group. Each class is shown in a separate panel. Bold lines (one for each treatment group) denote predicted class-specific trajectories. Thin lines denote observed 3MS scores over time for each individual in the study, color coded per treatment. For this figure, individuals were assigned to the class of which they were most likely to be a member. 3MS, Modified Mini-Mental State Examination.

The 3MS and DSRS classes and NSAID effects are comparable despite the differences in scale and scope of the two measurement instruments. For both measures, the three-class models were estimated, and the shapes of those trajectories (no decline, slow decline, and fast decline) were similar. Looking at the 4-year expected changes in both models, celecoxib had no effect on the no-decline class, a deleterious (but not significant for the 3MS) effect on the slow-decline class, and a substantial and statistically significant ameliorative effect on the fast-decline class. The results for naproxen were less clear. In both models, it had no effect on the no-decline class, but in the 3MS model, it was associated with a shallower downward slope relative to placebo for the slow-decline class, whereas in the DSRS model, it was associated with a steeper slope, although neither of these differences was statistically significant at 4 years. For the fast-decline class, naproxen was associated with worse outcome in both models, but this difference was only statistically significant in the 3MS model.

Discussion

Data from both the 3MS and the DSRS supported a three-class model: no decline, slow decline, and fast decline. We hypothesized that NSAID exposure would slow cognitive decline in the slow-decline group and speed cognitive decline in the fast-decline group, as compared with individuals on placebo. The results were not clear cut: naproxen seemed to confirm this hypothesis in the 3MS model, but in the DSRS model, although naproxen appeared harmful for the fast-decline class, it did not differ from placebo in the slow-decline class. Celecoxib showed a completely different pattern than expected: with both outcomes, it appeared harmful for individuals in the slow-decline class and helpful for individuals in the fast-decline class. In post-mortem studies of individuals at different stages of AD (both pre-clinical and post-clinical), neuronal COX-2 expression peaks early in the pre-clinical period and then decreases, whereas COX-1-expressing microglia are rarer early on but

Table 3 Predicted DSRS changes over 4 years per latent class and treatment group

	Class 1	Class 2	Class 3
Placebo	0.36 (−0.19, 0.91) ^a	1.81 (0.50, 3.12)	9.04 (5.64, 12.44)
Naproxen	0.32 (−0.57, 1.21)	2.2 (0.89, 3.51)	10.53 (6.98, 14.08)
Celecoxib	0.35 (−0.7, 1.4)	3.07 (2.02, 4.12)	4.19 (−0.09, 8.47)

$p = 0.40^b$ (Class 1, Placebo vs Naproxen)
 $p = 0.40$ (Class 1, Celecoxib vs Placebo)
 $p = 0.33$ (Class 2, Placebo vs Naproxen)
 $p = 0.02$ (Class 2, Celecoxib vs Placebo)
 $p = 0.33$ (Class 3, Placebo vs Naproxen)
 $p = 0.08$ (Class 3, Celecoxib vs Placebo)

DSRS, Dementia Severity Rating Scale.

^aPredicted change in DSRS score after 4 years for individuals in class 1 who were treated with placebo (95% CI).

^b*p*-value for the within-class comparison of predicted 4-year change in DSRS score on naproxen *versus* placebo.

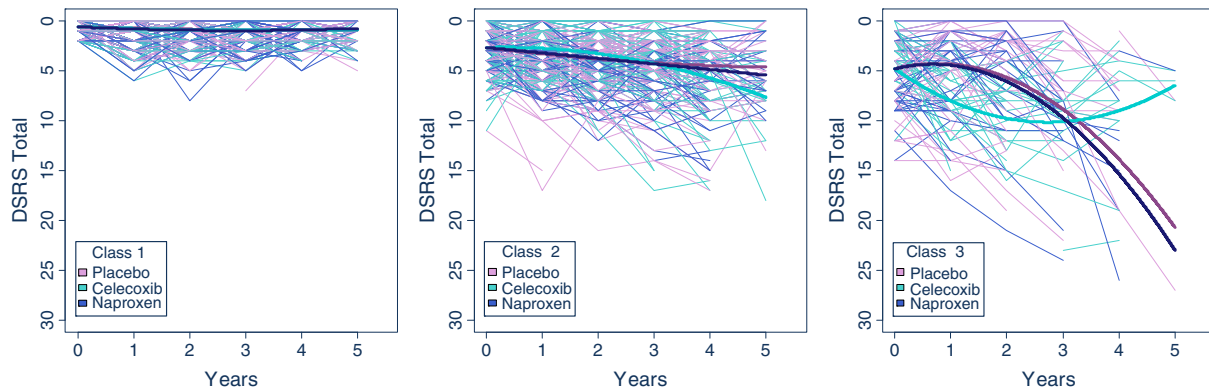


Figure 2 Fitted and observed DRS trajectories per latent class and treatment group. Each class is shown in a separate panel. Bold lines (one for each treatment group) denote predicted class-specific trajectories. Thin lines denote observed DRS scores over time for each individual in the study, color coded per treatment. For this figure, individuals were assigned to the class of which they were most likely to be a member. To facilitate comparison with Figure 1, the vertical axis for DRS has been reversed. DRS, Dementia Severity Rating Scale.

increase rapidly immediately prior to diagnosis (Hoozemans *et al.*, 2008, 2011). If these processes are adaptive rather than harmful, it would explain why COX-2 inhibition with celecoxib in the earlier (slow-decline) phase appeared harmful and why COX-1 inhibition by the non-specific COX inhibitor naproxen appeared harmful for individuals in the later (fast-decline) phase. Recent reports have shown that some NSAIDs (including celecoxib, but not naproxen) lower A β -42 levels, independent of their anti-inflammatory properties. However, a combined analysis of observational studies comparing NSAIDs with and without this property found no difference, although they did not stratify by pre-clinical disease stage or age (Szekely *et al.*, 2008b).

There are several strengths to our analyses. Limiting enrollment to individuals over 70 years of age with a family history of AD likely enriched the sample for pre-clinical AD. As an RCT, the timing, amount, and type of NSAID exposure are known and the results are not subject to the biases inherent in observational studies. The inclusion of both treatments allowed for differentiation between a COX-2 specific inhibitor and a non-specific COX1/COX2 inhibitor. Further, the use of GMMs is a significant advance over previous analyses that did not allow for NSAID effects to vary by pre-clinical phase. Apart from this, our analyses differ from previous ADAPT reports because the entire period of observation up to 5 years is included.

Several potential weaknesses related to the trial's design are noted. First, the results are not directly comparable with the bulk of findings from observational studies in which the most commonly used NSAIDs were aspirin and ibuprofen. Also, as treatment was halted because of safety concerns, and

enrollment occurred on a rolling basis, individuals were exposed to treatment for varying portions of the total time of observation. Although time on treatment did not vary significantly by treatment or class membership, there was a clear trend for shorter time on treatment for members of class 3 (fast decline). As treatment comparisons were made within rather than across classes, this is likely to have biased the results toward the null hypothesis by effectively decreasing the sample size in that class. Another potential issue is the assumption that all individuals experiencing cognitive decline are on an AD trajectory. Although the majority (67/75) of dementia cases have been adjudicated as AD, it is likely that some of these cases also have a vascular component, along with many individuals who have not yet received a clinical diagnosis. Less is known about the trajectory of individuals with vascular dementia, but it can be assumed that inclusion of individuals on a non-AD path would increase the "noise" within the sample, introduce uncertainty in class membership, and bias the results toward the null.

It is premature to make a clinical recommendation, but our findings point to several potential avenues of research. First, these results should be replicated in one or more existing large observational studies. Further observation of ADAPT participants will make it possible to model drug effects on AD-free survival as a function of latent trajectory class. Although more clinical trials are unlikely in the near-term, further *in vitro* and animal research into the role of inflammation and NSAIDs on AD pathogenesis is warranted, specifically with regard to differentiating which inflammatory processes are adaptive and which are potentially harmful and when during the pre-clinical period these

processes occur. Even if this work does not directly result in a treatment, it will have provided a valuable window on the pathophysiology of AD.

Conflicts of interest

Excerpts of an earlier version of this manuscript were presented during the poster session and Junior Investigator awardee presentation at the 10th Annual Meeting of the International College of Geriatric Psychoneuropharmacology in Athens, Greece on 15 September 2010.

Dr Leoutsakos has no conflicts of interest.

Dr Muthen is a developer and owner of MPLUS, the software used for the analyses in this paper.

Dr Breitner previously held partial royalty interests in two US patents for the use of NSAIDs for the prevention of AD; in January 2005, he assigned these interests irrevocably as a gift to a private charitable foundation in which he has no constructive or beneficial interest.

Dr Lyketsos is a consultant to GlaxoSmithKline and receives lecture honoraria and support for continuing medical education activities from AstraZeneca, Eisai, Forest Laboratories, Janssen, Lilly, Novartis, and Pfizer.

Pfizer provided celecoxib and matching placebo, and Bayer Healthcare provided naproxen sodium and matching placebo for the ADAPT trial.

Key points

- Heterogeneity in cognitive trajectories among individuals without dementia can be accounted for by the existence of phases of pre-clinical disease.
- The effects of NSAIDs on cognitive decline may vary as a function of the timing of exposure during the pre-clinical period.
- The effects of NSAIDs on cognitive decline may also vary as a function of the type of NSAID (COX-2 vs. non-specific).
- These findings suggest that inflammation plays a complex and dynamic role in the pathogenesis of Alzheimer disease.

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Appendix A. Growth mixture modeling

Longitudinal data from clinical trials are typically analyzed using either marginal or mixed effects regression models. Marginal models are appropriate in cases where one is interested in the population-averaged effects of treatment (Diggle *et al.*, 2002). By contrast, mixed effects models allow for the modeling of heterogeneity in both intercept and slope (Diggle *et al.*, 2002), thus acknowledging that individuals both start and end at different places, in terms of their outcome scores measured over time. Both of these approaches, though, carry the assumption that all individuals originate from the same population. In some cases, it is reasonable to hypothesize that a sample is comprised of individuals from more than one population. Acknowledging this is particularly important if treatment effects vary by population. In an extreme case, if a treatment were helpful in one group, but harmful in the other, analyses which ignored group membership would suggest (erroneously) that the treatment had no effect on longitudinal course. When group membership is known, separate analyses by group is a simple solution. However, when group membership is unknown, or *latent*, then group/class membership can be modeled using a mixture, or latent, class model. In fitting these models, it is assumed that each individual is a member of one and only one class, and that although class membership is unobserved, it can be inferred based on an individual's observed data. Standard latent class analysis is based on patterns of responses on categorical, typically dichotomous, observed variables. Recently, a longitudinal extension has been developed which groups individuals based on the shapes of their longitudinal trajectories (Muthén and Asparouhov, 2009). These GMMs have been increasingly used to model heterogeneity in development (Muthen *et al.*, 2002; Greenbaum *et al.*, 2005; Kreuter and Muthen, 2008) and to model class-specific treatment effects (Muthén and Brown, 2009). Adaptation for use in dementia research is a natural extension (Terrera *et al.*, 2009).

The simplest possible GMM has a single class. By letting y_{ij} represent the outcome measure for the i th person at the j th timepoint a_j , a trajectory can be modeled as $y_{ij} = I + Sa_j + Qa_j + \varepsilon_{ij}$ where I , S , and Q represent the fitted intercept, slope, and quadratic term, respectively. As written, this is a marginal or population-averaged model, because intercept, slope, and quadratic terms are the same for all individuals. If instead we wanted to allow these terms to vary by individual, we would write it as $y_{ij} = I_i + S_i a_j + Q_i a_j^2 + \varepsilon_{ij}$. Specifically, we want to allow these terms to vary as a function of class membership (indexed by k). We therefore write $I_i = \alpha_{I,k} + \zeta_{I,i}$, $S_i = \alpha_{S,k} + \zeta_{S,i}$

and $Q_i = \alpha_{Q,k} + \zeta_{Q,i}$, where $\alpha_{I,k}$, $\alpha_{S,k}$, and $\alpha_{Q,k}$ are class-specific intercept, slope, and quadratic terms, respectively.

Now we wish to add treatment effects, with treatment as a dichotomous variable T . With a simple marginal model, we could add two interaction terms: $y_{ij} = I + Sa_j + \gamma_S(T \times a_j) + Qa_j^2 + \gamma_Q(T \times a_j^2) + \varepsilon_{ij}$. The interpretation of the interaction terms γ_S and γ_Q are then the differences in slopes and quadratic terms, respectively, between those treated and untreated. To allow these terms to vary by class, we now include these interactions in the equations for S and Q and write $S_i = \alpha_{S,k} + \gamma_{S,k}T + \zeta_{S,i}$ and $Q_i = \alpha_{Q,k} + \gamma_{Q,k}T + \zeta_{Q,i}$.

With GMMs, it is also possible to model predictors of class membership. These variables are different from the longitudinal outcomes because they are conceptualized as causes, rather than effects, of latent class membership. Specifically, one models log odds of membership in a specific latent class, relative to a reference class. Typically, the largest class, or the one containing the most “normal” individuals, is chosen as the reference class.

The fitting of GMMs entails the estimation of a large number of parameters and assumptions regarding the variances of the intercept, slope, and quadratic terms for each class. Additionally, the number of classes that are estimated affects substantially the parameter estimates and their interpretation. Therefore, GMM models should be built in a careful and sequential manner. Jung and Wickrama (2008) provide an excellent and accessible guide to this process. First, the number of classes of trajectories to estimate should be decided by fitting simplified (e.g., without drug effects or predictors of class membership) models with numbers of classes ranging from 1 to 5 and examining Bayesian information criteria statistics, BLRTs, graphical model fit, and parameter estimates (Nylund *et al.*, 2007). If there is evidence of curvature in the trajectories, or an *a priori* hypothesis that such curvature will be present, then this process should be repeated with models which include quadratic terms. Models with and without quadratic terms can then be compared via likelihood ratio tests. If the p -value for the likelihood ratio test is less than 0.05, it can be taken as evidence that the model with fewer parameters (in this case, the model without the quadratic term) fits the data less well than the model with more parameters.

After determining an appropriate model for the observed outcome variables (Y_s), treatment effects can then be added. To determine if drug effects vary by latent class, one can compare, again via likelihood ratio test, two models: one in which the treatment effects are constant across all of the classes and one in which the treatment effects vary between classes. As a last step, predictors of class membership are added to the model. Figure 3 shows a schematic of the model estimated in this paper.

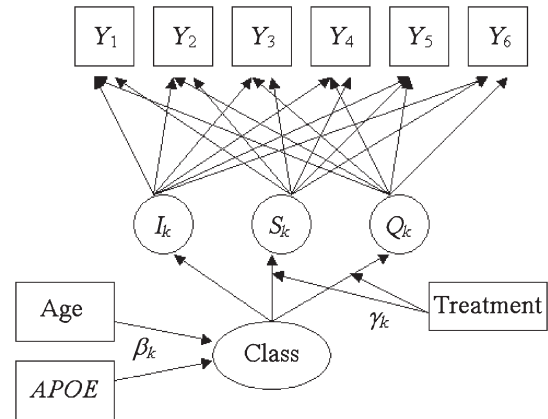


Figure 3 Schematic of growth mixture model.

In some cases, there is a need to make additional comparisons among the latent classes. One approach is to assign individuals to the class of which they are mostly likely to be a member and then stratify the analyses. However, this approach introduces bias in that it treats class membership as if it had been directly observed. A better approach is to multiply impute class membership and then combine the results across the imputations, producing a standard error estimate that takes into account the variability across the imputations (Bandeen-Roche *et al.*, 1997; Leoutsakos *et al.*, 2010).

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