Understanding the role of APOE Gene Polymorphisms in Minimal Atrophy Alzheimer's Disease by mixture of expert models

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Abstract—Alzheimer's disease (AD) is a heterogeneous disease. Exploring the characteristics of each AD subtype is the key to disentangling the heterogeneity. Minimal atrophy AD (MAD) is a common AD subtype that yields conflicting results. In order to evaluate this aspect across relatively large heterogeneous AD populations, a total of 192 AD and 228 cognitively normal (CN) subjects were processed by the automated segmentation scheme FreeSurfer, which generates regional cortical thickness measures. A machine learning driven approach, the mixture of expert models, which combines unsupervised modeling of mixtures of distributions with supervised learning of classifiers, was applied to approximates the non-linear boundary between AD and CN subjects with a piece-wise linear boundary. Multiple cortical thicknes patterns of AD were discovered, which includes: bilateral parietal/frontal atrophy AD, left temporal dominant atrophy AD, MAD, and diffuse atrophy AD. MAD had the highest proportions of ApoE4 and ApoE2. Further analysis revealed that ApoE genotype, disease stage and their interactions can partially explain the conflicting observations in MAD.

1 INTRODUCTION

As the most common type of dementia [1], Alzheimer's disease (AD) is expected to affect 1 out of 85 people in the world by the year 2050. It is widely believed to be triggered by extracellular senile plaques composed of amyloid β(Aβ) and intracellular neurofibrillary tangles (NFTs) [2]. As the understanding of AD has advanced, there is an increasing evidence of heterogeneity in the etiology, pathological changes, and pathogenesis of AD [3-5]. Investigators might question whether there exists one unifying pathophysiological process shared among AD patients, or multiple partly independent disease processes leading to a similar clinical syndrome.

Recent advances in neuropathological post-mortem studies greatly enhanced our understanding of the pathophysiology of AD subtypes. Based on density and the distribution of neurofibrillary tangles in three cortical regions and two hippocampal sectors, Murray et al. [6] classified AD subjects into hippocampal sparing AD, typical AD, and limbic predominant AD. Structural MRI measurements of regional brain atrophy have been shown to correlate well with the distribution and extent of neurofibrillary tangle pathology [7], which makes MRI a potential alternative marker of regional tangle distribution. Besides, the correlation between subtypes defined by neuropathology and structural MRI has been confirmed in the literature [8]. Machine learning approach based on MRI that explicitly models the heterogeneity of AD forms new perspectives to explain mechanism underlying pathophysiological processes of AD.

Structural AD subtypes are generally defined as the atrophic degree of the limbic system and four lobes. For example, Byun et al. [9] classified AD patients into four AD subtypes based on standardized values of hippocampal and regional cortical volumes. Three identified subtypes correlate well with neuropathological findings of neurofibrillary tangle distribution, a fourth subtype is identified with no atrophy or minimal atrophy. This subtype is commonly known as minimal AD (MAD). Subjects may have clinical symptoms, but with no obvious changes in brain structure compared with normal aging. While mild to severe atrophy may support the diagnosis of AD, the significance of isolated minimal or mild atrophy in MAD remains unclear.

Table I provides a brief comparison of brain regions in MAD. Evidences from several studies [14-16] show early onset of MAD. However, a study by Byun et al. [10] yields conflicting results. Although some investigators [10,14] report that the MMSE score of MAD is the best among all subtypes, one study [13] finds that it is the worst. The proportion of Apolipoprotein E (ApoE) ε4 carriers in MAD are the highest in several studies [14-16], but it is the lowest in multiple independent studies [11,13]. Abnormal amyloid-beta$_{1\text{-}42}$ (A$\beta_{1\text{-}42}$) carriers account for the smallest proportion in Ten Kate's research [16], but the opposite result is reported in other researches [10,14]. The statistical results of tau also have generated mixed results. To be specific, the proportions of both total tau (T-tau) and phosphorylated tau$_{181P}$ (P-tau) are the highest in some

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researches [14,16], but others report that they are closer to normal aging [10,11].

Lacking of atrophy in MAD questions the value of atrophy in MRI based AD diagnosis. Moreover, mixed results of MAD also attract researches' interests. Emrani et al. [17] found that ApoE4 is not only an important mediator of AD susceptibility, but it likely confers specific phenotypic heterogeneity in AD presentation. There is a fundamental divergence in AD manifestation related to ApoE genotype. We hypothesize that conflicting observations of MAD could be partially explained by the proportion of ApoE genotype. In this study, a recently developed mixture of experts (MOEs) method [18], which combines unsupervised clustering modelling with supervised learning of classifiers is applied to identify multiple AD subtypes. After that, a detailed analysis is performed on MAD subjects to uncover the possible reasons for those inconsistencies.

Table1. AFFECTED BRAIN REGIONS IN MAD

Study	Name and prevalence	Affected brain regions
Byun et al. [10] (2015)	both spared（10.4%）	Do not have any prominent atrophy in either hippocampal or association cortical regions
Dong et al. [11] (2017)	mild or none; non-focal（30.35%）	generally normal anatomy
Park et al. [12] (2017)	diffuse atrophy (28.4%）	sporadic atrophy over the cortices
Persson et al. [13] (2017)	minimal atrophy (13%)	Do not have any prominent atrophy in medial temporal lobes, the frontal regions, the frontal regions, etc.
Ferreira et al. [14] (2018)	minimal atrophy（16.8%）	mainly located in brain lobar areas
Poulakis et al. [15] (2018)	minimal atrophy（18.1%）	minimal brain atrophy in the left entorhinal cortex
Ten Kate et al. [16] (2018)	mild atrophy（37.1%）	dispersed pattern of cluster-defining regions, amongst the motor cortex

2 MATERIALS AND METHODS

2.1 Participants

The data used in the preparation of this article was obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI) [19-21]. Launched in 2004, ADNI phase one is an international project that collects and validates neurological data, such as MRI and PET images, genetics or cognitive tests. About 800 subjects, age at 55-90 years, including CN (cognitive normal) individuals, patients with AD and mild cognitive impairmentt (MCI) were recruited. 192 AD (age 75.4±7.4 years; education 16.1±2.9 years and MMSE 29.1±1.0) and 188 CN participants (age 75.9±5.0 years; education 14.7±3.1 years and MMSE 23.3±2.0) was used in the study. Baseline cerebrospinal fluid (CSF) T-tau, P-tau were available for approximately one-half of subjects. Of 228 CN participants, 111 had CSF data available. Of 192 AD participants, 98 had their Aβ1-42 and P-tau data available, and 96 had CSF T-tau.

2.2 MRI processing

Structural T1-weighted MRI were acquired using 1.5 T scanners. The cortical reconstruction and the volume segmentation pipelines in FreeSurfer software package (http://freesurfer.net/) include motion correction, brain tissue extraction, affine transformation, segmentation of subcortical white matter (WM) and deep GM volumetric structures, tessellation of the white surface, topology correction, and surface deformation. The anatomical thickness from cortical and subcortical labeling pipelines based on Desikan-Killiany atlas was provided by ADNI website, which can be used for subsequent analyses.

2.3 MOEs based AD subtype identification

If not properly controlled, extraneous variables may cause undesired subtype differences. The effect of age, sex, years of education, and intracranial volume were removed from the model. CN subjects served as the reference group, while AD subjects served as the affected group, within which to identify multiple directions of deviation from CN. MOEs analysis combines unsupervised clustering with supervised SVM classification, and a piece-wise linear boundary is generated to approximate the non-linear discriminating boundary. Thus, it identifies multiple groups within the heterogeneous AD group, as well as multivariate patterns that discriminate each AD subtype from the CN [22]. Five-fold cross validation was performed in the MOEs to evaluate algorithm performance.

2.4 Statistical analyses

Statistical analyses were performed using SPSS Statistics, version 19.0, and the significance level was 95%. The demographic variables, cognitive outcome measures, ApoE status, and CSF biomarker levels were compared across AD subtypes. Quantitative variable comparisons were analysed by one-way analysis of variance (ANOVA) with Dunnett test as a post-test. The chi-square test was used only for qualitative variables. Abnormal proportions were calculated with missing data excluded.

3 RESULTS

Based on the MOEs approach, AD participants were divided into four subtypes with a reasonable separation from NC. The average accuracy was 83.1±4.8%. Four subtypes are the followings: (1) Bilateral parietal/frontal AD (PFAD, n=56, 29.2%), with atrophy prominent in bilateral parietal and frontal. (2) Left temporal dominant AD (LTAD, n=43, 22.4%), with atrophy prominent in the left lateral parietal, middle, and inferior temporal. (3) MAD (n=31, 16.1%), with the least atrophy (4) Diffused AD (DAD, n=62, 32.3%), with atrophy in almost all cortical regions. No significant differences were found among four subtypes in age, years of education, or vital characteristics. The characteristics of the four AD subtypes were compared in Table II.

The MAD had lowest FAQ scores in four subtypes. The proportions of ApoE4 and ApoE2 carried in MAD were the highest, where the proportions of ApoE2 were significantly different from other subtypes. In the quantitative analysis of $A\beta_{1\text{-}42}$, the concentration of $A\beta_{1\text{-}42}$ in MAD was significantly higher than PFAD and DAD, and their abnormality ratio was the lowest. The concentration and abnormality ratios of T-tau and P-tau in MAD were lowest. The abnormality ratios of T-tau and P-tau in MAD were significantly different from those in PFAD through the qualitative analysis.

Table2. DEMOGRAPHIC、COGNITIVE AND NEUROPATHOLOGICAL CHARACTERISTICS OF THE STUDY SUBTYPES

Characteristics	PFAD	LTAD	MAD	DAD	P values (MAD only)
n (%)	56 (29.2%)	43 (22.4%)	31 (16.1%)	62 (32.3%)	-
MMSE	23.9±1.9	24.0±2.0	24.0±1.8	22.6±2.1	＜0.001 [c]
CDR-SB	4.2±1.5	4.0±1.4	3.8±1.5	4.8±1.8	0.011[c]
FAQ	12.6±6.3	11.9±6.4	8.9±5.6	15.9±7.1	＜0.001 [abc]
ApoE4 (n(carry%))	38(67.9%)	27(62.8%)	22(71.0%)	40(64.5%)	0.962 [d]
heterozygote	29(51.8%)	19(44.2%)	16(51.6%)	27(43.5%)	-
homozygote	9(16.1%)	8(18.6%)	6(19.4%)	13(21.0%)	-
ApoE2 (n(carry%))	3(5.4%)	1(2.3%)	7(22.6%)	3(4.8%)	0.018 [abcd]
$A\beta_{1\text{-}42}$ (abnormal%)	31(97.0%)	21(84.0%)	10(76.9%)	26(93.9%)	0.151 [d]
T-tau (abnormal%)	25(78.1%)	17(68.0%)	4(30.8%)	18(54.5%)	0.014 [ad]
P -tau (abnormal%)	30(93.8%)	23(92.0%)	8(61.5%)	30(90.9%)	0.015 [ad]

[a] Significant differences between PFAD and MAD. [b] Significant differences between LTAD and MAD.

[c] Significant differences (P<0.05) between MAD and DAD. [d] The χ^2 test was used.

4 DISCUSSIONS AND CONCLUSIONS

In this study, we identified four AD subtypes based on MOEs algorithm. The subjects in MAD share similar cognitive and neuropathological characteristics as some previous studies, such as better cognitive performance [10,14], higher concentration of and lower abnormality ratio of Aβ1-42 [16], higher proportions of ApoE4 [14-16], and lower T-tau, P-tau concentration and abnormality ratios [10-11].

The highest proportions of ApoE4 and ApoE2 in MAD play a crucial role in interpretation of related results. ApoE is a 34 kDa lipid-binding protein which was discovered in very-low-density lipoprotein in 1973. ApoE is a component of a key modulator of lipoprotein, plasma lipoprotein, and cholesterol concentrations. In human, ApoE has three isoforms, ApoE2, ApoE3, and ApoE4, which are expressed by the polymorphic alleles: *ε2*, *ε3*, and *ε4*. ApoE2 has been recognized as a neuroprotective variant for AD [23,24]. One investigator [25] report that ApoE2 is associated with lower atrophy rate relative to ApoE3 in aged people. Our results showed that MAD patients with ApoE2 carriers preserved cortical structures better over time. In the follow-up study, those ApoE2 carriers had the highest proportion (4/6 and 3/4 in 12 and 24 months) to maintain their subtype. Among the three polymorphic alleles, ApoE4 is the most important genetic risk factor of AD. Therefore, MAD patients with ApoE4 may be diagnosed at early stage of disease, and have younger disease onset age. This inference has been confirmed in our study, ApoE2 heterozygote has older onset age (77.1 years) than ApoE4 homozygote and heterozygote (70.03 and 75.44 years). Moreover, ApoE4 homozygote and heterozygote also demonstrate worse neuropathological consequences (T-tau(ng/L) 96.7, 86.0, and P-tau (ng/L), 37.7, 32.0) than ApoE2 heterozygote (T-tau(ng/L) 84.7, P-tau (ng/L) 27.0) in MAD. All MAD patients with ApoE4 homozygote progressed to other AD subtype during 24 months. ApoE2 carrier can tolerate more AD pathology in the early stage of the AD, so neuropathological consequences appear better. However,

when the pathology was more advanced, this protective effect would be masked. The differences of gene inheritance, disease stage and their interactions will result in different experimental results in MAD, so more attentions are needed for those factors in MAD related study.

ACKNOWLEDGMENTS

This research was financially supported by grants from National Natural Science Foundation of China (81971683) and Natural Science Foundation of Beijing Municipality (L182010).

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