

Propositional Density and Apolipoprotein E Genotype among Persons at Risk for Familial Alzheimer's Disease

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Key Words

Presenilin 1 · Amyloid precursor protein · Early-onset Alzheimer's disease · Preclinical dementia · Alzheimer's disease · Linguistic ability

Abstract

Background/Aims: A relationship between decreased propositional density (p-density) in young adulthood and future risk for Alzheimer's disease (AD) has been postulated, but multiple interpretations of the nature of this relationship are possible. This study explored the relationship between familial AD (FAD) mutation status, apolipoprotein E (*APOE*) genotype, and p-density. **Methods:** Thirty-five non-demented persons at risk for FAD mutations were recruited. Subjects wrote brief biographical essays from which p-density, the ratio of the number of unique ideas to the number of words in the text, was calculated. Mixed-effects regression models were used to examine the relationship of p-density and FAD mutation status and *APOE* genotype. **Results:** FAD mutation status was not significantly associated with p-density. However, results from both models indicated that the presence of the *APOE* $\epsilon 4$ allele was significantly associated with p-density ($p < 0.0001$), with *APOE* $\epsilon 4$ carriers hav-

ing lower p-density than non-carriers. **Conclusions:** Our results are consistent with an influence of *APOE* status on p-density in young adulthood that is independent of the AD risk per se and suggest the previous finding of increased risk for the development of AD in persons with decreased p-density may be related to *APOE* genotype.

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The Nun Study identified a connection between literacy in early life and memory decline in late life [1] and helped elaborate the idea of cognitive reserve in Alzheimer's disease (AD). Cognitive reserve, a hypothesized protective factor against cognitive deficits later in life, posits that relevant abilities make the brain resistant to manifesting clinical symptoms in the face of developing AD or other neuropathology [2]. By relating archival essays written by nuns (mean age of 22 years) to cognitive function and neuropathological findings five to seven decades later, Snowdon et al. [1] found a link between decreased propositional density, or p-density, and neuropsychological deficits and AD neuropathology [3] later in life. P-density, first used by Kintsch and Keenan [4] as a measure of retention, is defined as the number of in-

dependent ideas embedded within a certain number of words in written text. Compared to other measures of linguistic ability (e.g. grammatical complexity), p-density has been shown to be relatively more resistant to normal aging processes, but sensitive to AD pathology, which leads to a rapid decline in p-density [5, 6].

AD is the most common cause of dementia and affects nearly 5.4 million Americans [7]. In addition to early-life literacy and other factors related to cognitive reserve (e.g. educational and occupational attainment), risk factors include age, traumatic brain injury, cardiovascular risk factors, and the presence of the apolipoprotein $\epsilon 4$ allele (*APOE* $\epsilon 4$). Unlike these risk factors, which merely influence the likelihood that one might develop AD, autosomal dominant mutations in the presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), and amyloid precursor protein (*APP*) genes are essentially determinant for the development of AD pathology at a relatively early age [8]. Persons at risk for inheriting this form of familial AD (FAD), in whom the genetic alteration has been present since conception, provide a unique opportunity to study the earliest changes related to the disease. Our prior studies have suggested a trend towards lower educational attainment in carriers of *PSEN1* and *APP* mutations [9] and studies in Colombian persons at risk for the E280A *PSEN1* mutation found diminished production of semantic units when describing a visual scene in preclinical mutation carriers (mean age, mid 40s) [10].

In the current study, we sought to replicate the finding of reduced p-density in persons destined to develop dementia in a preclinical Mexican and Mexican-American population at risk for inheriting FAD. We hypothesized that FAD mutation carriers (MCs) would exhibit lower p-density than non-carriers (NCs) when taking into account factors such as age, education, language of essay, acculturation, country of residence, and *APOE* status.

Methods

Study Population

Thirty-five participants were recruited. Of these, 30 were at risk for mutations in the *PSEN1* gene, 1 for the *PSEN2* gene, and 4 for the *APP* gene by virtue of being first-degree relatives of demented persons identified to have such a pathogenic mutation. All were at least 18 years of age and, at the time of the study, had Clinical Dementia Rating (CDR) scores of <1.0 (see below). All provided written informed consent, and all procedures were approved by the Institutional Review Boards at UCLA and the National Institute of Neurology and Neurosurgery in Mexico City.

Procedures

Genetic testing was performed on all participants to determine whether they were FAD MCs or NCs. The presence of mutations in *PSEN1* and *PSEN2* was assessed using restriction fragment length polymorphism analyses. The presence of mutations in *APP* was assessed with direct sequencing. *APOE* genotyping was performed using standard techniques. The CDR [11] was performed with an unrelated informant and with the subject. This structured interview rates asymptomatic persons as 0, persons with questionable cognitive impairment as 0.5, and mild, moderate, and severe stages of dementia are rated as 1, 2, and 3 respectively. Subjects were also administered the Cognitive Abilities Screening Inventory (CASI) [12] and completed a questionnaire quantifying degree of acculturation to the United States [13]. Both the individuals and the investigators were blind to mutation status, except in the case of one presymptomatic MC who had undergone clinical testing. As in the original Nun Study [1], participants were given the following prompt in both English and Spanish:

Write a short sketch of your own life. This account should not contain more than two to three hundred words and should be written on a single sheet of paper. Include your place of birth, parentage, interesting and edifying events of your childhood, schools attended, religious life, and its outstanding events.

Participants wrote essays in the language with which they felt most comfortable (i.e. English or Spanish). Essays were then analyzed for p-density by 3 raters, fluent in both English and Spanish, who were blind to all clinical and genetic information in accordance with methods described by Snowden et al. [1] using the last 10 sentences of each essay. Inter-rater reliability for all three raters had a Cronbach's α value of 0.93. Mean p-density score was used in statistical analyses.

Statistical Analysis

Although the age of onset in FAD tends to be consistent within families, it can vary between families [14]. Thus, an 'adjusted age' is calculated for each subject relative to the median age of disease diagnosis in their families. This allows for comparisons to be made between subjects with regard to proximity to the onset of FAD. Additional factors of interest included educational level, age at enrollment, gender, CDR score, country of residence, language of essay, and acculturation level. We compared each of these factors between FAD MCs and NCs as well as among *APOE* genotypes using ANOVA. Next, a mixed-effects regression model with family-level random effects was used to assess the association between genetic status (i.e. FAD mutation, *APOE* $\epsilon 4$ status) and p-density. The model included a family-level random intercept to account for dependence within families, which helps to avoid under-estimation of the fixed effect variance and increases efficiency in identifying important sources of variation [15]. Two mixed-effects regression analyses, unadjusted (Model 1) and adjusted (Model 2) for the predictors of interest, were performed. Values of $p \leq 0.05$ were considered significant. All statistical analyses were performed using STATA, version 9.1.

Table 1. Profile of the sample

	Entire sample	Mutation status		APOE		
		MC	NC	$\epsilon 2/3$	$\epsilon 3/3$	$\epsilon 4/3$
Subjects, n	35	22	13	6	24	5
Age at enrollment, years	34.9 ± 8.9	31.9 ± 6.8**	40.1 ± 9.8**	34.0 ± 5.8	33.5 ± 8.7	45.0 ± 8.6
Adjusted age, years	-10.3 ± 10.9	-13.4 ± 8.0	-5.2 ± 13.4	-8.3 ± 7.5	-12.1 ± 10.3	-2.3 ± 16.8
Education, years	12.6 ± 3.2	12.4 ± 2.7	13.0 ± 3.9	13.5 ± 3.8	12.4 ± 3.1	12.4 ± 3.2
Acculturation score	-1.4 ± 1.9	-1.3 ± 1.7	-1.6 ± 2.2	-2.2 ± 0.52	-1.2 ± 2.1	-1.0 ± 1.4
CASI score	91.35 ± 5.8	89.8 ± 6.6	93.8 ± 3.2	91.7 ± 4.3	90.5 ± 6.4	95.5 ± 1.0
CDR score = 0.5, n	7	7	0	1	6	0
FAD MCs, n	22	NA	NA	5	15	2
p-density	0.70 ± 0.04	0.70 ± 0.04	0.69 ± 0.03	0.69 ± 0.04**	0.70 ± 0.04**	0.64 ± 0.03**
Residing in the USA, n	19	12	7	0**	16**	3**
Essay written in English, n	13	8	5	0	11	2
Female, n	26	16	10	5	18	3

Data are presented as means ± SD, unless indicated otherwise. ** $p < 0.01$, all p values are derived from Model 2.

Results

MCs ($n = 22$) were significantly younger than NCs ($n = 13$) in both age at enrollment ($M_{MC} = 31.9$, $M_{NC} = 40.1$; $p = 0.006$) and age relative to expected disease onset ($M_{MC} = -13.4$, $M_{NC} = -5.2$; $p = 0.028$). There were no significant differences between MCs and NCs with regard to gender, years of education, country of residence, language of essay, acculturation score, or total CASI score. Distribution of APOE status among MCs and NCs was not statistically different. Mean p-density of the sample ($M = 0.70$, $SD = 0.04$) was comparable to means originally reported by Snowdon et al. [1] ($M = 0.70$, $SD = 0.12$).

Among the 35 subjects, 24 had the $\epsilon 3/3$, 6 the $\epsilon 2/3$ genotype, and 5 the $\epsilon 4/3$ APOE genotype. There were no differences between APOE genotype groups in age at enrollment, proximity to the onset of FAD, gender, years of education, language of essay, acculturation score, or total CASI score. However, those with the $\epsilon 2/3$ APOE genotype were more likely to reside in Mexico than those with the $\epsilon 3/3$ or $\epsilon 4/3$ genotypes ($p = 0.008$; table 1).

No significant associations between p-density and proximity to the diagnosis of FAD (i.e., adjusted age), age at enrollment, CDR score, years of education, country of residence, language of essay, acculturation score, or mutation status were observed. Additionally, p-density did not differ significantly between the three FAD genes

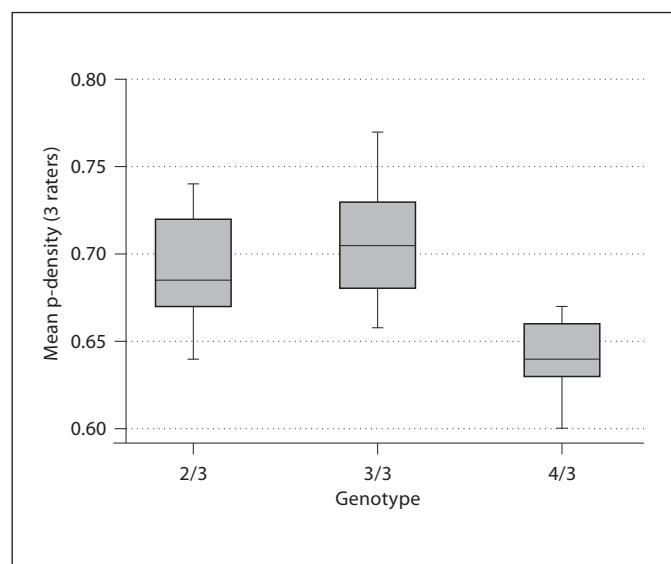


Fig. 1. Mean p-density by APOE genotype.

studied (i.e., *PSEN1*, *PSEN2*, and *APP*). However, results from both unadjusted and adjusted models indicated that the presence of the APOE $\epsilon 4$ allele was significantly associated with p-density ($p < 0.0001$) with the presence of the APOE $\epsilon 4$ allele being associated with lower p-density (fig. 1).

Discussion

Contrary to our expectations, no differences were observed in p-density between carriers and non-carriers of FAD mutations. However, individuals who were *APOE* $\epsilon 4$ positive had significantly lower p-density scores than those who were *APOE* $\epsilon 4$ negative, regardless of their FAD mutation status. These results held when accounting for educational level, CDR score, language of essay, acculturation score, and age relative to the typical age of the family-specific age of AD diagnosis.

In light of the findings in the Nun Study and the study by Cuetos et al. [10], we anticipated that preclinical FAD MCs would have a lower p-density than NCs. The population in the latter study, in which carriers of the E280A *PSEN1* mutation produced fewer semantic categories during verbal description of the Cookie Theft Scenario, was slightly older (43 vs. 35 years) and had less variability in age (SD = 3.1 vs. 8.9 years) than our population. It is therefore possible therefore that the changes they found were evidence of incipient neurodegeneration and the lack of differences between FAD MCs and NCs in our study was related to the fact many carriers were quite young in relation to the age of disease onset. Alternatively, differences in the sensitivity of the specific language measures employed or levels of education between the two populations (5.1 vs. 12.6 years in our study) could account for the disparate findings.

APOE is a pleiotropic molecule and diverse mechanisms through which it contributes to AD risk have been proposed, including effects on development [16]. Although the original Nun Study paper did not examine literacy in relation to *APOE*, our finding of an association between the *APOE* $\epsilon 4$ genotype and p-density in sub-clinical persons is consistent with prior studies demonstrating subtle impairment of language abilities in *APOE* $\epsilon 4$ carriers. Such studies have found non-demented carriers of the *APOE* $\epsilon 4$ genotype to have poorer perfor-

mance on object naming and semantic fluency than non-carriers [17] and persons with Down syndrome with the *APOE* $\epsilon 4$ allele had poorer language function than those without the *APOE* $\epsilon 4$ allele [18].

As none of the carriers of the *APOE* $\epsilon 4$ allele in our study were symptomatic (all had CDR scores of 0, CASI scores were non-significantly higher than the other groups), our results are not accountable for by the presence of the early stages of progressive dementia. However, the relatively small number of subjects in our study makes it susceptible to random effects and, therefore, interpretations should be made with caution. Nonetheless, our results are consistent with an effect of *APOE* status on p-density in early adulthood that is independent of AD risk per se and suggest that the previously reported association of reduced p-density and subsequent risk for AD may be related to *APOE* genotype.

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Disclosure Statement

No authors have relevant conflicts of interest. L.D.M. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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