FINAL REPORT

Impact of APOE genotype on cardiorespiratory fitness in aging and AD

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This manuscript discusses the study on cardiorespiratory fitness under the impact of cognitive impairment and genetic Alzheimer's disease(AD) risk. The clinical objective is to achieve more effective fitness therapies towards Alzheimer's disease patients, based on the statistical profiling on current patient data. Given the data collected from two Alzheimer's disease centers (University of Kansas Medical Center and University of Wisconsin), we make insight on some of the categorical variables such as gender, diagnosis and APOE genotype, and explore the selection of linear regression models that characterize the relations between maximal oxygen consumption (VO_2 max) as a response and other predictor variables under interest.

KEYWORDS:
cardiorespiratory fitness; Alzheimer's disease (AD); VO_2; E_4; APOE; Age; Gender;

1 | INTRODUCTION

An estimated 5.3 million Americans have Alzheimer's disease(AD) and the prevalence is expected to double by mid-century.[1,2] There are currently no disease-modifying or preventive treatment for AD.[3,2] However observational evidence in human suggests higher levels of cardiorespiratory fitness and physical activity are associated with greater brain volumn, less brain atrophy, slower dementia progression and reduced risk of dementia.[4,5,6,7,2,8,9,10] Although current evidence remains insufficient to conclude that exercise is an effective therapeutic for AD or cognitive decline,[3,11,2] exercise continues to be a promising area of research for AD treatment.[2,12].

Our primary objective was to characterize the impact of cognitive impairment and genetic AD risk on maximal (VO_2 max) and sub-maximal(OUES) measures of cardiorespiratory fitness. For this report, we focus only on VO_2.

2 | METHOD

2.1 | Participants

Participants were recruited as part of intervention and observation studies at the University of Kansas Alzheimer's Disease Center(KU) and the Wisconsin Alzheimer's Disease Research Center(UW). We have previously reported results from these investigations.[4,13,2] Briefly, both sites are part of the US network of Alzheimer's disease centersof excellence that support research into brain aging and dementia.

The KU sample was drawn from both intervention and observation studies (ClinicalTrials.gov: NCT01129115, NCT02000583, NCT01128361, NCT00267124). All individuals were evaluated using the Clinical Dementia Rating (CDR) and determined to have either normal cognition or cognitive impairment due to early-stage Alzheimer's disease (CDR 0.5 or 1). Individuals also comleted a graded maximal exercise test, which is taken at baseline.

All individuals from UW sample were evaluated using the CDR and determined to have normal cognition (no AD, or denoted as ND in the data).

2.2 | Inclusion Criteria

For the current investigation we pooled the most recent graded maximal CR fitness tests(GXT) of each individual.[14] CR fitness was indexed as the maximal oxygen uptake during the GXT normalized to whole body mass ($ml*kg^{-1}*min^{-1}$). Oxygen uptake efficiency slope(OUES) was calculated by regressing oxygen uptake on the log transformed total ventilation at each 15s sampling period of the entire GXT.[15] OUES provides an estimation of cardiorespiratory efficiency. Only individuals who achieved a respiratory exchange ratio(RER) $\geq$ 1.1 were included in the final analysis. Initial dataset has 510 participants from both centers, and 304 participants were included in the analysis.

2.3 | Analysis

All analyses were performed using R version 3.5.1. For those non default R packages that are used here: the "foreign" package is used to read in the data from the initial format of SPSS; the "ggplot2" and "gridExtra" packages are used to generate all the plots; the "dplyr" package is used to transform data from wide format to long format when needed; the "gmodel" package is used to report Pearson χ^2-test and Fisher's exact test for contingency tables of categorical variables; the "olsrr" package is used to do stepwise/forward/backward selection of linear regression models, and the "multcomp" package is used to make inferences on combinations of regression coefficients.

We first investigate three dichotomous variables of interest, that are gender (female = 1, male = 0), diagnosis(DX)(AD = 1, ND = 0) and E4carrier(Carrier = 1, Noncarrier = 0). We perform Pearson χ^2-test and Fisher's exact test pairwisely to test on

associations. We also stratify on each one of the three variables, and perform Breslow-Day test on the other two for common odds ratio. If fail to reject, we perform Cochran-Mantel-Haenszel test for conditional independence.

We then build linear regression models with VO_2 max as the response, and discuss model selections. For continuous predictor variables, age and body mass index(BMI) are considered. For categorical variables, we consider gender, diagnosis, site(UW = 1, KU = 0) and E4 carrier and possible two way interactions between those categorical variables. Later we also consider other APOE genes (E2, E3, E4 and their combinations). The statistical methods we use include stepwise/forward/backward selections and lack of fit test. We aim to identify the most parsimonious model that is adequate for the current data, while during model selection we try to find and interpret information from subgroup comparisons.

3 | RESULTS

Gender / DX	ND	AD	Row Total
Male	84	46	130
Female	135	39	174
Column Total	219	85	304

Pearson χ^2	df	p	Fisher's exact test	
Pearson χ^2 test			estimate odds ratio:	0.529
6.215	1	0.013	H_a : odds ratio < 1	
Pearson χ^2 with Yate's continuity correction			p	0.009
5.588	1	0.018	95% CI	$(0, 0.833)$

TABLE 1 Gender vs Diagnosis

From Table 1 we conclude that diagnosis is related to gender(χ^2- $p = 0.01$, Fisher exact $p = 0.01$), and male has a smaller odds of not having cognitive impairment, or in other words, male has higher odds of developing AD.

Gender / E4	Noncarrier	Carrier	Row Total
Male	82	48	130
Female	91	83	174
Column Total	173	131	304

Pearson χ^2	df	p	Fisher's exact test	
Pearson χ^2 test			estimate odds ratio:	1.556
3.525	1	0.060	H_a : odds ratio > 1	
Pearson χ^2 with Yate's continuity correction			p	0.039
3.100	1	0.078	95% CI	$(1.028, +\infty)$

TABLE 2 Gender vs E4

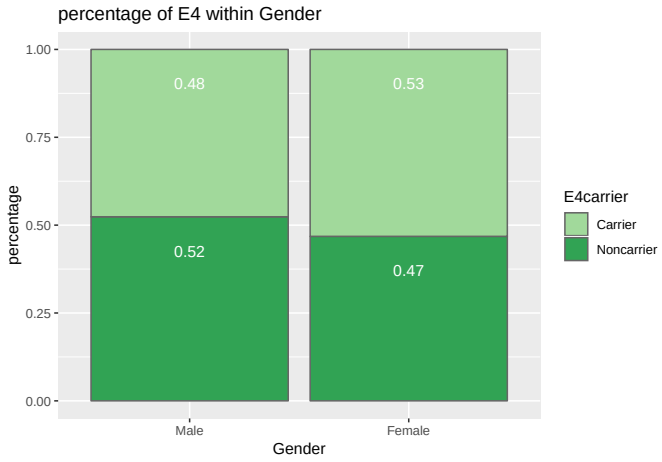

FIGURE 1 percentage of E4carrier from UW

From Table2 the pearson χ^2 test suggests that E4 carrier is independent from gender ($p = 0.06$), while Fisher's exact test suggests that female has a larger odds of carrying E4 allele ($p = 0.04$). The two tests are telling different stories, and the relationship between the two variables are indeterminant.

We also make further comments on the above observations:

(a) Fisher's exact test needs the assumption that the row and column total are pre-fixed. In our investigation this is not really satisfied and we just 'abused' Fisher's exact test.

(b) Notice that all the UW individuals are without cognitive impairment(hence, with ND), however among these individuals larger proportion of females(53%) are E4 carrier, and larger proportion of males(52%) are not E4 carrier.(See figure 1). Also we have more females(47) than male(21) from that center. This supports the Fisher exact test in Table2 that female has a higher odds of carrying E4 allele, but is not in consistency with the result in Table3 below, that E4 carrier has higher odds of developing AD. Then a question here would be, should we include data from UW in our further analysis given that all participants there are younger people without cognitive impairment? We will investigate this question below when it comes to model selection.

From table3 we conclude that DX(diagnosis) is related to E4 allele (both χ^2 and Fisher exact p values < 0.001). E4 carrier has a much higher odds of developing AD (estimated odds ratio 2.783), which meets common sense.

When we stratify on each of gender, diagnosis, and E4 carrier variables, Breslow-Day tests return a p value of 0.1 for each case, accommodating the null hypothesis of common odds ratio. A further test of Cochran-Mantel-Haenszel says that DX is related to E4 when conditioning on gender($\chi^2_1 = 17.38, p < 0.001$); gender is related to E4 when conditioning on DX($\chi^2_1 = 5.79, p = 0.02$); DX is related to gender when conditioning on E4($\chi^2_1 = 8.32, p = 0.004$)

Diagnosis / E4	Noncarrier	Carrier	Row Total
ND	140	79	219
AD	33	52	85
Column Total	173	131	304

Pearson χ^2	df	p	Fisher's exact test	
Pearson χ^2 test			estimate odds ratio:	2.783
15.736	1	< 0.001	H_a : odds ratio > 1	
Pearson χ^2 with Yate's continuity correction			p	< 0.001
14.729	1	< 0.001	95% CI	$(1.752, +\infty)$

TABLE 3 Diagnosis vs E4

For model selection, we first fit simple linear regression model and ANOVA model for individual predictors including Age($p < 0.001$), BMI($p < 0.001$), Gender($p < 0.001$), DX($p = 0.003$), Site($p < 0.001$) and E4carrier($p = 0.28$). All are significant except for E4 gene carrier.

We then take VO_2 as the response and consider the following reduced(main effect) and complete(with interaction) models:

$$\mathrm{VO}_2 = \beta_0 + \beta_1 \cdot \mathrm{Age} + \beta_2 \cdot \mathrm{BMI} + \beta_3 \cdot \mathrm{Gender} + \beta_4 \cdot \mathrm{DX} + \beta_5 \cdot \mathrm{Site} + \beta_6 \cdot \mathrm{E4(\ Reduced\ )}$$

$$\begin{aligned}\mathrm{VO}_2 = \beta_0 + \beta_1 \cdot \mathrm{Age} + \beta_2 \cdot \mathrm{BMI} + \beta_3 \cdot \mathrm{Gender} + \beta_4 \cdot \mathrm{DX} + \beta_5 \cdot \mathrm{Site} + \beta_6 \cdot \mathrm{E4} \\ + \beta_7 \cdot \mathrm{Gender} \cdot \mathrm{DX} + \beta_8 \cdot \mathrm{Gender} \cdot \mathrm{E4} + \beta_9 \cdot \mathrm{DX} \cdot \mathrm{E4(\ Complete\ )}\end{aligned}$$

For the main effect model, a stepwise/forward/backward selection procedure will yield a more parsimonious model as

$$\mathrm{VO}_2 = \beta_0 + \beta_1 \cdot \mathrm{Age} + \beta_2 \cdot \mathrm{BMI} + \beta_3 \cdot \mathrm{Gender} + \beta_4 \cdot \mathrm{DX}$$

Compare between the main effect model($R^2_{\text{adj}} = 0.520$) and the complete model($R^2_{\text{adj}} = 0.524$), a lack-of-fit F test will suggest for the reduced model($F_{3,295} = 1.786, p = 0.150$).

From a lack-of-fit point of view, the reduced model is adequate to fit our data. Also from the selection methods based on p values, we seem to only need a more parsimonious main effect model as mentioned above. However the complete model is not entirely useless. With interaction terms between three dichotomous variables(gender, DX, and E4), we could make inferences on the comparisons of the $2^3 = 8$ subgroups.

For example, we may be interested in the female participants who do not carry E4 allele, and want to compare the VO_2 max performance between AD and ND cases in this subgroup. This is equivalent to making inference on $H_0 : \beta_4 + \beta_7 = 0$ from the complete model above. Our conclusion for this case is that the difference is not significant (p = 0.4).

Figure 2 Shows a scatter plot between the illustrated two groups. We argue that since there are only 9 samples for group (female, AD, NonE4), the above inference may lack power.

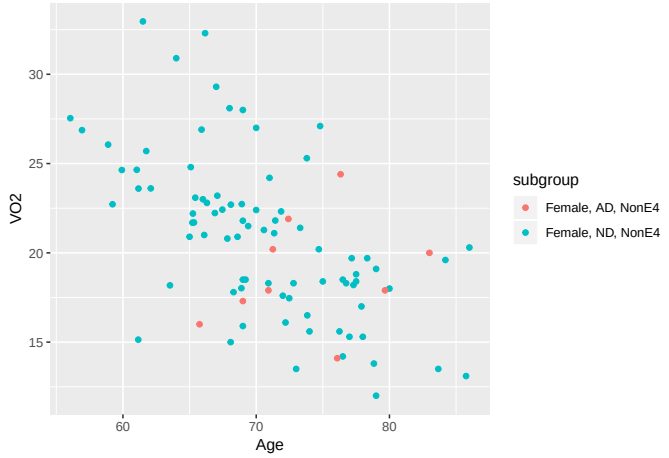

FIGURE 2 AD vs ND for noncarrier females

A complete summary of tests on all the subgroup comparisons are given below in Table 4 and the scatter plots are given in Figure 3 , 4 , 5

AD vs ND		Female vs Male		E4 vs NonE4	
	p		p		p
(male, nonE4)	0.004	(ND, nonE4)	< 0.001	(male, ND)	0.34
(male, E4)	< 0.001	(ND, E4)	< 0.001	(male, AD)	0.1
(female, nonE4)	0.36	(AD, nonE4)	< 0.001	(female, ND)	0.5
(female, E4)	0.04	(AD, E4)	< 0.001	(female, AD)	0.7

TABLE 4 VO2 performance on subgroups

From Table 4 we conclude that, among all subgroups there is a significant VO2 difference between AD and ND, except for those who are females and non E4 carriers ($p = 0.36$). As mentioned above that due to the low sample size of group (female, AD, NonE4) (only 9 participants), this result lacks power. We also observe a significant VO2 difference on gender. This makes sense since in theory the males should have a higher VO2 scores than the females. Finally we do not observe any VO2 difference between E4 and non E4 carriers, among all subgroups.

We are concerned about the fact that all participants from WX site are younger people without AD, such that may create impact on our conclusion. So we did an independent analysis on the KS site only.

The categorical variables under the interest are the same as before, namely, gender, diagnosis and E4 carrier. The categorical analysis give pretty much the same results as before, which we skip the details here.

As for model selection, the complete and reduced models are

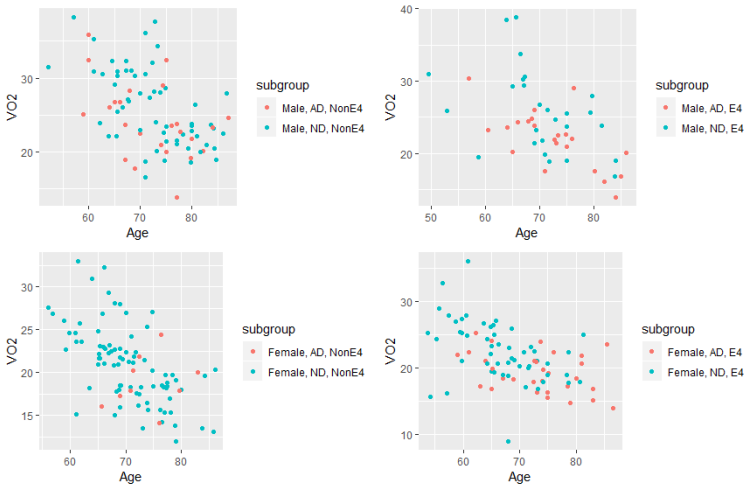

FIGURE 3 AD vs ND

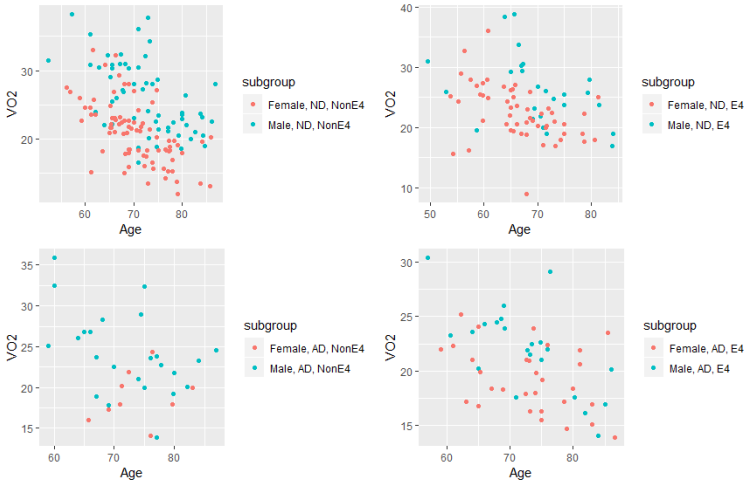

FIGURE 4 Female vs Male

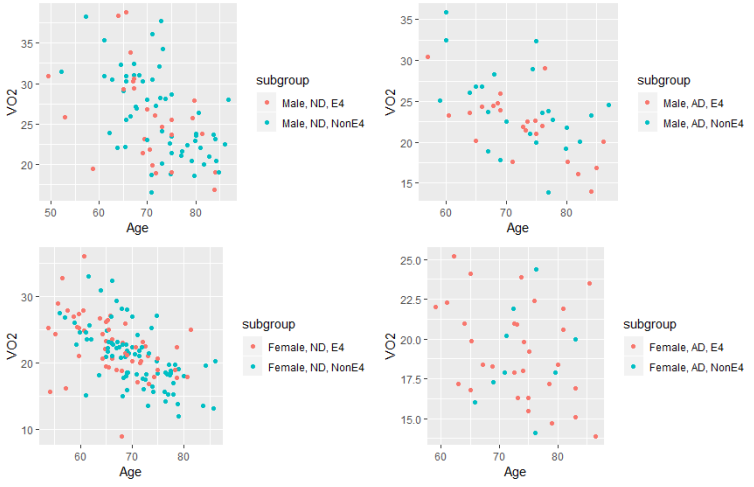

FIGURE 5 E4 vs NonE4

$$\text{VO}_2 = \beta_0 + \beta_1 \cdot \text{Age} + \beta_2 \cdot \text{BMI} + \beta_3 \cdot \text{Gender} + \beta_4 \cdot \text{DX} + \beta_5 \cdot \text{E4(Reduced)}$$

$$\text{VO}_2 = \beta_0 + \beta_1 \cdot \text{Age} + \beta_2 \cdot \text{BMI} + \beta_3 \cdot \text{Gender} + \beta_4 \cdot \text{DX} + \beta_5 \cdot \text{E4} + \beta_6 \cdot \text{Gender} \cdot \text{DX} + \beta_7 \cdot \text{Gender} \cdot \text{E4} + \beta_8 \cdot \text{DX} \cdot \text{E4(Complete)}$$

Site is no longer included since we are only using data from KS site.

A subgroup analysis from the complete model yields Table 5 :

AD vs ND		Female vs Male		E4 vs NonE4	
	p		p		p
(male, nonE4)	0.008	(ND, nonE4)	< 0.001	(male, ND)	0.42
(male, E4)	0.003	(ND, E4)	< 0.001	(male, AD)	0.1
(female, nonE4)	0.39	(AD, nonE4)	< 0.001	(female, ND)	0.66
(female, E4)	0.05	(AD, E4)	< 0.001	(female, AD)	0.66

TABLE 5 VO2 performance on subgroups in KS

Comparing to Table 4 that uses data from both sites, the p values have minor changes but the significance results are almost identical at 0.05 level. The only one that gets a different significance result is between AD vs ND in the subgroup of females and E4 carriers. p values have changed from 0.04 to 0.05, and hence the conclusion goes from 'significant' to 'marginally significant'. We do not regard this as a major concern. The previous p value is already close to 0.05 threshold, and the current one is not a big change either.

An F test for lack of fit suggests the reduced model ($F_{3,227} = 1.443$, $p = 0.23$) and the stepwise/forward/backward selection on the main effect reduced model suggests the same parsimonious final model as before:

$$\text{VO}_2 = \beta_0 + \beta_1 \cdot \text{Age} + \beta_2 \cdot \text{BMI} + \beta_3 \cdot \text{Gender} + \beta_4 \cdot \text{DX}$$

In conclusion, keeping or taking away the data from WX site will not change the model selection and subgroup analysis, and to boost power we would prefer to use data from both sites.

To further explore the potential impact by different APOE gene types, we refine our model based on the APOE allele combination, instead of just using E4 carrier as a dichotomous indicator.

There are in total six different allele combinations based on $E2$, $E3$ and $E4$ these three alleles (See Table 6):

Here $E2$ is an allele that is 'protective' against AD development, E3 is 'neutral', and E4 is 'detrimental'. So the idea is to assign different scores to these allele, and assess the contribution of each combination to the AD status.

APOE	E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4
Count	5	28	8	140	106	17

TABLE 6 Count of APOE allele Combinations

A natural approach would be: $E4 = -1$, $E3 = 0$ and $E2 = 1$. The higher the score is, the more 'protective', or less risky it is to develop Alzheimer's disease. Thus the combination of "$E3/E3$" and "$E2/E4$" will be categorized under the same group due to the same score as 0, and all other different combinations form their individual groups.

Treating the allele combinations grouped by scores as an ordinal categorical variable, a Cochran-Armitage test shows a decreasing trend between AD and allele scores ($p < 0.001$)

The refined main effect regression model is (referenced on $E3/E3$ and $E2/E4$ group)

$$\begin{aligned} \mathrm{VO}_2 = \beta_0 + \beta_1 \cdot \mathrm{Age} + \beta_2 \cdot \mathrm{BMI} + \beta_3 \cdot \mathrm{Gender} + \beta_4 \cdot \mathrm{DX} + \beta_5 \cdot \mathrm{Site} \\ + \beta_6 \cdot I(\mathrm{alleles} = -2) + \beta_7 \cdot I(\mathrm{alleles} = -1) + \beta_8 \cdot I(\mathrm{alleles} = 1) + \beta_9 \cdot I(\mathrm{allele} = 2) \end{aligned}$$

There can be different, but equivalent parametrizations if we use different allele groups as reference. Under the above parametrization, the p values for allele groups are (β_6: 0.15, β_7: 0.76, β_8: 0.65 and β_9: 0.48), which says the allele combinations are not significant in predicting VO_2 scores.

A lack-of-fit test suggests for the reduced main effect model($F_{4,294} = 0.75$, $p = 0.56$).

4 | DISCUSSION

We explored the current Alzheimer's disease data and discussed relationships between categorical variables under interest. We also tried to look for parsimonious but informational regression models to help us profile the significant predictor variables for VO_2 performance. We suggests the final model to be a main effect model including age, body mass index, gender, diagnosis and alzheimer's disease status as predictor variables. Unfortunately we did not see any impact or contribution of E4 allele carrier to the VO_2 max score, nor do we see any interaction between these categorical variables. After taking a closer look at APOE genotypes, we do not seem to find any impact of these genotypes to the VO_2 max scores either.

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