PLOS Medicine

Apolipoprotein E4 is associated with improved cognitive function in Amazonian forager-horticulturalists with a high parasite burden

--Manuscript Draft--

Manuscript Number:	PMEDICINE-D-16-02863
Full Title:	Apolipoprotein E4 is associated with improved cognitive function in Amazonian forager-horticulturalists with a high parasite burden
Short Title:	Cognitive Function, APOE4 and Parasite Burden
Article Type:	Research Article
Keywords:	antagonistic pleiotropy; mismatch; cognitive decline; Tsimane; Alzheimer's Disease
Corresponding Author:	Benjamin Trumble Arizona State University UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Arizona State University
Corresponding Author's Secondary Institution:	
First Author:	Benjamin Trumble
First Author Secondary Information:	
Order of Authors:	Benjamin Trumble
	Jonathan Stieglitz
	Blackwell Aaron
	Hooman Allayee
	Bret Beheim
	Caleb E Finch
	Michael Gurven
	Hillard Kaplan
Order of Authors Secondary Information:	
Abstract:	The Apolipoprotein E4 ('E4') allele is present worldwide, despite its associations with higher risk of cardiovascular morbidity, accelerated cognitive decline during aging, and Alzheimer's disease. The E4 allele is especially prevalent in some tropical regions with high parasite burden. Equatorial populations also face a potential 'dual-burden' of high E4 prevalence combined with parasitic infections that can also reduce cognitive performance. Here we examine interactions between E4, parasite burden and cognitive performance in a traditional, non-industrialized population of Amazonian forager-horticulturalists (N=372) to test whether E4 protects against cognitive decline in environments with heavy pathogen burden. Contrary to observations in industrial populations, older adult E4 carriers with high parasite burdens either maintained, or showed slight improvements in cognitive performance, while non-E4 carriers with a high parasite burden showed reduced cognitive performance. While being an E4 carrier is the strongest risk factor to date of AD and cognitive decline in industrial populations, it is associated with greater cognitive performance in individuals facing a high parasite and pathogen load, suggesting advantages to the E4 allele under certain environmental conditions. The current mismatch between post-industrial hygienic lifestyles and active parasite-rich environs may be critical for understanding genetic risk for cognitive ageing.
Suggested Reviewers:	Daniel Eisenberg, PhD

	University of Washington dtae@dtae.net Previous publications on global distribution of APOE http://www.ncbi.nlm.nih.gov/pubmed/20734437
	Rudi GJ Westendorp, MD, PhD Professor, University of Copenhagen westendorp@sund.ku.dk Expertise on APOE and ageing
	Douglas Ewbank, phD University of Pennsylvania ewbank@pop.upenn.edu Expertise on APOE and ageing
Opposed Reviewers:	
Additional Information:	
Question	**Response**
Financial Disclosure Please describe all sources of funding that have supported your work. **This information is required for submission and will be published with your article, should it be accepted.** A complete funding statement should do the following: Include **grant numbers and the URLs** of any funder's website. Use the full name, not acronyms, of funding institutions, and use initials to identify authors who received the funding. **Describe the role** of any sponsors or funders in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. If the funders had **no role** in any of the above, include this sentence at the end of your statement: "*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*" However, if the study was **unfunded**, please provide a statement that clearly indicates this, for example: "*The author(s) received no specific funding for this work.*" * typeset	National Institutes of Health (NIH)/ National Institute on Aging (NIA) (R01AG024119, R56AG024119) The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Competing Interests You are responsible for recognizing and disclosing on behalf of all authors any competing interest that could be perceived to bias their work, acknowledging all financial support and any other relevant financial or non-	"The authors have declared that no competing interests exist."

financial competing interests. Do any authors of this manuscript have competing interests (as described in the PLOS Policy on Declaration and Evaluation of Competing Interests)? **If yes**, please provide details about any and all competing interests in the box below. Your response should begin with this statement: *I have read the journal's policy and the authors of this manuscript have the following competing interests:* **If no** authors have any competing interests to declare, please enter this statement in the box: "*The authors have declared that no competing interests exist.*" * typeset	
Data Availability PLOS journals require authors to make all data underlying the findings described in their manuscript fully available, without restriction and from the time of publication, with only rare exceptions to address legal and ethical concerns (see the PLOS Data Policy and FAQ for further details). When submitting a manuscript, authors must provide a Data Availability Statement that describes where the data underlying their manuscript can be found. Your answers to the following constitute your statement about data availability and will be included with the article in the event of publication. **Please note that simply stating 'data available on request from the author' is not acceptable. *If, however, your data are only available upon request from the author(s), you must answer "No" to the first question below, and explain your exceptional situation in the text box provided.*** Do the authors confirm that all data underlying the findings described in their manuscript are fully available without restriction?	No - some restrictions will apply
Please describe where your data may be found, writing in full sentences. **Your answers should be entered into the box**	Data are from the Tsimane Health and Life History Project whose authors may be contacted at https://www.unm.edu/~tsimane/.

below and will be published in the form you provide them, if your manuscript is accepted. If you are copying our sample text below, please ensure you replace any instances of XXX with the appropriate details. If your data are all contained within the paper and/or Supporting Information files, please state this in your answer below. For example, “All relevant data are within the paper and its Supporting Information files.” If your data are held or will be held in a public repository, include URLs, accession numbers or DOIs. For example, “All XXX files are available from the XXX database (accession number(s) XXX, XXX)." If this information will only be available after acceptance, please indicate this by ticking the box below. If neither of these applies but you are able to provide details of access elsewhere, with or without limitations, please do so in the box below. For example: “Data are available from the XXX Institutional Data Access / Ethics Committee for researchers who meet the criteria for access to confidential data.” “Data are from the XXX study whose authors may be contacted at XXX.” * typeset	
Additional data availability information:	

school of human evolution & social change
ARIZONA STATE UNIVERSITY

Dear Editors,
Enclosed please find our manuscript "Apolipoprotein E4 is associated with improved cognitive function in Amazonian forager-horticulturalists with a high parasite burden" which we are submitting for consideration in the special issue on "*Dementia across the lifespan and around the globe—Pathophysiology, prevention, treatment, and societal impact".*

To our knowledge this is the first study to examine associations between genetics, cognitive aging, and biomarkers of parasite burden in a large sample of pre-industrial forager-horticulturalists practicing a traditional lifeway. We feel that makes this paper ideally suited for this special issue, which specifically asks for "Genetic and environmental risk factors for dementia across geographical settings, time, age groups, and unique populations".

While being a carrier of the ApoE4 allele is the single strongest genetic risk factor for Alzheimer's disease and cognitive decline in industrial populations, here we find evidence that it is associated with improved cognitive performance in individuals with a high parasite and pathogen burden. Parasites also generally result in poorer cognitive performance worldwide, but in this case we find evidence that ApoE4 carriers are able to maintain cognitive function despite high parasite burden. This may help explain the persistence of such an otherwise costly allele.

Studies in industrial populations indicate that individuals homozygotic for the ApoE4 allele have over a 90% chance of developing Alzheimer's dementia by age 80. Beyond Alzheimer's disease, being a carrier of ApoE4 also increases the risk of cardiovascular disease and high cholesterol. Despite the risk of morbidity and mortality associated with carrying the ApoE4 allele, it is present in 14.5% of people worldwide, and in some populations >45% of individuals are carriers. In particular, there is an especially high prevalence of the ApoE4 allele in tropical regions where helminths and other endoparasites are endemic.

While helminths and other parasites currently impact millions around the world, improved sanitation and public health campaigns have significantly reduced parasite burdens, particularly in industrialized populations where most studies of cognitive aging have focused. However the modern lifestyles in such populations are an evolutionary novelty; >99% of human history occurred in small mobile hunter gatherer groups, without access to sanitation or clean water. The current mismatch between modern hygienic lifestyles and active parasite-rich environs may be critical for understanding the evolution of genetic risks for cognitive ageing.

These results are of interest to a broad range of researchers and the public, including physicians, geneticists, gerontologists, psychologists, and evolutionary biologists, and thus we think this manuscript would be ideally suited for this call for papers on dementia and cognitive aging. The data here represent the largest dataset combining genetics biomarkers, and cognitive functioning among a remote indigenous population ever collected. As indigenous populations are rapidly becoming more market-integrated and rapidly gain access to medical care, these data may also represent one of our last opportunities to study cognitive ageing under natural-fertility and high pathogen conditions.

Thank you for your consideration,

Benjamin Trumble, PhD
Assistant Professor, Arizona State University
School of Human Evolution and Social Change & Center for Evolution and Medicine

School of Human Evolution and Social Change
an academic unit of the College of Liberal Arts and Sciences
PO Box 872402 Tempe, AZ 85287-2402
(480) 965-6215 Fax: (480) 965-7671
shesc.asu.edu

Apolipoprotein E4 is associated with improved cognitive function in Amazonian forager-horticulturalists with a high parasite burden

Trumble, Benjamin C[1,2]

Stieglitz, Jonathan[3,4]

Blackwell, Aaron D[5]

Allayee, Hooman[6]

Beheim, Bret[7]

Finch, Caleb E[8]

Gurven, Michael[5]

Kaplan, Hillard[4]

[1]School of Human Evolution and Social Change, Arizona State University, Tempe AZ

[2]Center for Evolution and Medicine, Arizona State University, Tempe AZ

[3]Institute for Advanced Study in Toulouse, Toulouse, France

[4]Department of Anthropology, University of New Mexico, Albuquerque NM

[5]Department of Anthropology, University of California Santa Barbara, Santa Barbara CA

[6] Department of Preventive Medicine and Institute for Genetic Medicine, Keck School of Medicine, University of Southern California, Los Angeles

[7]Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

[8] Davis School of Gerontology, University of Southern California, Los Angeles

Key Words: antagonistic pleiotropy, mismatch, cognitive decline, Tsimane, Alzheimer's Disease

Abstract

The Apolipoprotein E4 (‘E4’) allele is present worldwide, despite its associations with higher risk of cardiovascular morbidity, accelerated cognitive decline during aging, and Alzheimer**’s** disease. The E4 allele is especially prevalent in some tropical regions with high parasite burden. Equatorial populations also face a potential ‘dual-burden’ of high E4 prevalence combined with parasitic infections that can also reduce cognitive performance. Here we examine interactions between E4, parasite burden and cognitive performance in a traditional, non-industrialized population of Amazonian forager-horticulturalists (N=372) to test whether E4 protects against cognitive decline in environments with heavy pathogen burden. Contrary to observations in industrial populations, older adult E4 carriers with high parasite burdens either maintained, or showed slight improvements in cognitive performance, while non-E4 carriers with a high parasite burden showed reduced cognitive performance. While being an E4 carrier is the strongest risk factor to date of AD and cognitive decline in industrial populations, it is associated with greater cognitive performance in individuals facing a high parasite and pathogen load, suggesting advantages to the E4 allele under certain environmental conditions. The current mismatch between post-industrial hygienic lifestyles and active parasite-rich environs may be critical for understanding genetic risk for cognitive ageing.

Introduction

The strongest reported genetic risk factor of Alzheimer's disease (AD) is the Apolipoprotein E4 allele ('E4'), which raises lifetime risk up to four-fold compared to non-carriers [1-5]. Women are particularly vulnerable, showing a >50% excess of AD over men, and greater levels of brain amyloid and other markers of neurodegeneration [6]. Beyond increased AD risk, E4 carriers display a sharper rate of cognitive decline and of grey matter atrophy with synapse loss during normal adult aging compared to non-carriers [7]. In addition to cognitive decline and dementia risks, E4 is also associated with higher total cholesterol and low density lipoprotein (LDL), enhanced intestinal absorption of dietary cholesterol [8, 9] and increased cardiovascular disease (CVD) risk in industrialized populations [10-12]. Since E4 contributes to extensive morbidity and mortality worldwide [13, 14]; from an evolutionary perspective, the relatively high worldwide frequency of the E4 allele despite its costs to survivorship is somewhat puzzling. E4 is considered the ancestral allele in the genus *Homo* [15-18], and E4 frequencies show extensive geographical variation, from <5% to >45%, with higher frequencies at the equator and tropical latitudes, and in northern Europe [15, 19]. Tsimane fall in the upper range with 24-32% E4 across age groups [44].

Despite its strong association with AD risk in industrialized populations, the E4 allele might have conferred survival or reproductive benefits in response to parasite burdens in energy-limited subsistence-level populations. In high parasite environments, E4 appears to be protective against parasites by minimizing their deleterious impacts, e.g. by preventing or contributing to spontaneous clearance of some infections including viral Hepatitis C [20] and giardia and cryptosporidium in an urban slum [21-23], and cryptosporidium infection in a murine model with human APOE4 alleles [2]. Infectious disease burden impacts cognitive performance [24-26]

through a variety of mechanisms including malabsorption of macro and micronutrients from the parasite feeding directly on the host (and host gut contents), as well as increased host inflammatory burden and immune response to the parasitic infection [27]. While helminths and other parasites currently impact millions around the world, improved sanitation and public health campaigns have significantly reduced parasite burdens, particularly in industrialized populations, which are the best studied for cognitive aging. But the modern lifestyles in such populations are an evolutionary novelty; >99% of human history occurred in small, mobile hunter-gatherer groups without access to sanitation or clean water. The current mismatch between modern hygienic lifestyles and active parasite-rich environs may be critical for understanding the evolution of genetic risks for cognitive ageing.

Cognitive performance plays a crucial role in school achievement, job attainment, income, and social status, all of which contribute to reproductive fitness [28, 29]. Cognitive ability has been critical to the success of the human evolutionary niche, which requires extractive foraging, skill-intensive hunting and social intelligence [30, 31]. Adaptations that benefit cognitive performance via resistance to parasites or pathogens could have important reproductive impacts in early or later life. The reproductive benefits of cognitive performance could occur during early reproductive life with an earlier age at first reproduction, or at later ages as post-reproductive older adults enhance the reproductive success of descendants [32, 33]. Given that the E4 allele is present in less than half the individuals in any one population, it is possible that there is a heterozygotic advantage in populations with a high infectious burden such that individuals with only one E4 allele have highest fitness.

Current Study

While two studies have reported positive associations between E4 and child cognitive development [34, 35], it is not known whether E4 confers cognitive protection to older adults with a high parasite load. Studies thus far have not taken a life course perspective (either focusing only on children or only examining cognitive decline and AD in older adults), nor have there been adequate tests in populations more representative of human evolutionary past. From a mechanistic standpoint, the impact of E4 on cholesterol production and absorption and parasite/pathogen clearance should have the same impact on adults as children. Here we test two hypotheses: (H1) the E4 allele interacts positively with parasite burden in predicting cognitive function in adults; versus (H2): the positive early-life benefits of carrying E4, in interaction with parasite burden, with respect to brain development and cognitive performance is limited to childhood and adolescence [22, 34, 35], but could outweigh the purely deleterious consequences of E4 in causing more rapid decline in cognitive performance later in life, when the force of selection is reduced [36]. H2 predicts that cognitive advantages from E4 are limited to children (H2:P1), whereas fitness-relevant disadvantages (poorer cognitive performance) should be observed among post-reproductive adults (H2:P2). These hypotheses are tested among the Tsimane, a remote population of forager-horticulturalists (approximately 15,000 individuals) in the Bolivian Amazon who live a traditional lifestyle of small-scale horticulture, hunting, fishing and gathering without access to sanitation, electricity or running water [37]. The Tsimane have a high rate of pathogen exposure, with over two-thirds of adults showing active helminth infections [38, 39].

Methods

Tsimane participants (n=372 from 28 villages) aged 6-88 years (mean age 37.2 years, 51.6% male) participated in a series of cognitive tasks and a morning fasting blood draw as part of routine biomedical surveillance conducted annually by the Tsimane Health and Life History Project, see Figure S1. Ages were estimated from a combination of known ages from written records, relative age lists, photo-comparisons and dated events; Spanish speaking ability was self-reported on a three point scale (minimal to none, intermediate, fluent), and years of schooling were also collected. [40]. All participants provided informed consent, and the procedures were approved by the institutional review boards at the University of California-Santa Barbara and University of New Mexico.

Biomarkers of Parasitic Burden

A manual leucocyte count and five-part differential were collected immediately after a blood-draw, and erythrocyte sedimentation rate (ESR) was assessed using the Westergren Method [38]. Eosinophil counts measured via manual five part differential were used as an index of parasitic infections as they relate to both current and history of multiple types of parasitic infections [41, 42]. While hookworm, which induces Th-2 response characterized by eosinophilia [42] is the most common helminth seen in 56% of Tsimane [38, 39], eosinophilia is provoked by parasitic infections of many different types; instead of focusing on single species of parasite eosinophils are used as a biomarker of parasitic infection more generally. Previous studies report that quantitative measures of helminth burden in fecal samples are only marginally associated with clinical symptoms [43]. ESR and leucocyte counts were included as non-specific measures of inflammation and infection, respectively.

Cognitive Battery

Participants engaged in a seven part cognitive battery assessing attention, psychomotor speed, verbal declarative memory and semantic fluency derived in part from the Mexican Healthy Aging Study [44, 45]). The first task examined short and long term verbal memory via word recall; participants were asked to listen to and repeat a list of eight Tsimane words immediately and again after 10 minutes. A second set of tasks assessed working memory using three digit span tasks; participants were asked to repeat a series of digits in both Tsimane and Spanish language that increased in length until failure on two consecutive trials. While not all Tsimane speak Spanish, all Tsimane understand Spanish numerals. In the third digit span task (tactile), the interviewer touched a sequence of numbered boxes on a sheet of paper; participants were asked to touch the boxes in the same order. The next task examined semantic memory (category fluency) with three locally salient categories: animals, plants, and fish. Participants generated a list of items from memory that matched each category (e.g. all animals) during a two minute period. The final task was a visual scan where participants were shown a random array of symbols, and asked to highlight all instances of a target symbol. All tests were conducted by a trained Tsimane translator at participants' homes and in the Tsimane language, with the exception of the Spanish numbers in the digit forward test. Twelve individuals who reported vision problems were removed from analyses for tasks requiring visual acuity (Figure S1).

Genotyping

Whole blood was frozen in liquid nitrogen before transfer on dry ice to the University of Southern California where DNA was extracted using standard protocols. Determination of the

APOE2/E3/E4 alleles in the Tsimane derived from genotypes of two SNPs, rs429358 and rs7412 [38, 46]. Genotyping was performed using the TaqMan Allelic Discrimination system (Life Technologies, Carlsbad, CA).

Data analysis

Age, sex, schooling, and Spanish fluency are all associated with cognitive outcomes [44, 45], and are included as control variables in linear regression models. Community ID was used as a random effect in all analyses. All cognitive measures were transformed to z-scores to facilitate effect size comparisons across the distinct cognitive tasks. Analyses are divided into two sub-sets based on the hypothesis of interest: 1) all individuals over age 30 to study cognitive aging (previous studies in industrialized populations and among Tsimane show cognitive decline beginning in the 20s [44]) as specified by H1; and 2) the subset of the sample who are under age 18, and a second subset of post-reproductive adults (aged 45+) to test H2.

Results

E4 and biomarkers of infection

Among the study participants, the frequencies of APOE genotypes in the Tsimane were E3/E3=76.1%, E3/E4=21.3%, and E4/E4=2.6%, and the distribution of genotypes was in Hardy-Weinberg equilibrium ($\chi^2 = 2.34$, p=0.31). Approximately one quarter (23.9%) of the subjects carried at least one copy of E4 but, notably, the E2 allele is not present in the Tsimane. Given the low frequency of E4/E4 homozygotes, these individuals were grouped together with E4 heterozygotes for all genetic analyses.

For all adults aged 30+, eosinophil counts ranged from 61 cells/uL to 3400 cells/uL, with a mean count of 1282 cells/uL (SD ± 644); 85.9% of participants presented with eosinophilia (eosinophil count >600 cells/uL [47]). For adults aged 30+, E4 carriers had 20% lower eosinophil counts (β= -259 cells/uL, p=0.006), and 9% lower leucocyte counts (β= -723 cells/uL, p=0.031), after controlling for age and sex, with a random effect for community of residence. There was no association between E4 and ESR. By comparison, eosinophil counts among children under age 18 ranged from 110 cells/uL to 6604 cells/uL, with a mean ± SD count of 1676 ± 1164 cells/uL; 88.8% of children presented with eosinophilia. E4 status was not associated with eosinophil counts, leucocyte counts or ESR in any age group.

Does E4 protect cognitive function in a high parasite environment? (H1)

For adults aged 30+ (n=242, mean age 49.3 years, 50.8% male), E4 was associated with poorer cognitive performance on most measures of cognitive performance, especially for measures of fluid cognition (Table 1 Model 1, Table S1) after controlling for age, sex, education, Spanish language ability, and parasite burden (eosinophil count). However, there was a strong significant interaction between E4 and parasite burden (Figure 1, Table 1 Model 2, Table S1). Without including the interaction, the main effects of the E4 allele were weak and non-significant; after adding the interaction to the model, there was both a strong negative association between cognitive performance and the E4 allele and a strong positive interaction between E4 and eosinophils (Figure 1). The interaction was strong enough that with a high pathogen burden E4 carriers were expected to outperform E3 carriers on cognitive measures (see Figures 1 & Figure 2 A-F).

In the absence of eosinophilia, E4 carriers recalled 16 % fewer words in short-term tasks (p=0.004), 13% fewer words in long-term recall tasks (p=0.047), and 12% fewer repeated

numerals in the Tsimane digit forward task (p=0.024). There were also non-significant trends among E4 carriers for recalling 10% fewer animals, plants and trees in the category fluency task (p=0.096), and 8% poorer performance on the composite digit span task (p=0.068), combined Spanish, Tsimane and spatial digit span tasks). Eosinophilia consistently had protective effects against E4, with significant interactions for short (p=0.001) and long term recall (p=0.013), and trends toward an interaction for the Tsimane digit span (p=0.097),a composite digit span (p=0.051), and composite fluid task (p=0.004); the other cognitive measures all had positive interactions between E4 and eosinophils but were not statistically significant (Table S1, Figures 2A-F). There was no significant interaction between sex and E4 status.

Is E4 associated with improved cognitive performance among children? (H2:P1)

Controlling for age, sex, education and Spanish fluency, E4 was positively associated with visual scan (p=0.037) and spatial forward tasks (p=0.032), and positively (though not significantly) associated with 6/8 cognitive outcomes (Table S2) for 124 children aged 6-18 (mean age 11.9 years, 49.6% male). Height-for-age and weight-for-age Z scores were positively, but not significantly associated with E4 (Table S2). Unlike adults, there was no interaction between E4 and parasite load for cognitive outcomes (Table S3, Figure S2A-F).

Do post-reproductive E4 carriers show reduced cognitive performance? (H2:P2)

Limiting the sample to post-reproductive adults aged 45-88 (n=138, 51.5% male, mean age 58.2) produced results similar to those of all adults aged 30+. There was a consistent negative association between E4 and cognitive performance at lower eosinophil counts, whereas E4 was protective among eosinophilia cases (Table S4, Figures 3A-F).

Discussion

Extensive evidence in human population samples and in animal models has shown positive associations between parasite infections and cognitive impairment [24-26, 48]. Genotypes that mitigate the negative impacts of parasite burden on cognitive performance should therefore have a selective advantage. Here we present evidence that the E4 allele, often associated with cognitive decline, AD, and CVD in industrialized populations with minimal parasite burden, was associated with higher cognitive function among Tsimane Amerindians, but only among individuals with evidence of high parasite burden. For homozygous E3/E3 carriers, higher eosinophil counts are associated with poorer performance on all cognitive measures (see Table 2). However, adults with high eosinophil counts, indicative of high parasite burden, who carried at least one copy of the E4 allele (E3/E4 or E4/E4) showed better cognitive performance compared to non-carriers. E4 carriers also showed significantly lower eosinophil counts, suggesting potentially protective effects of E4 against parasitic infection or burden, consistent with previous reports [2, 20, 22]. This implies that E4 may mitigate the effects of pathogen burden through at least two routes: one by lowering parasite load itself and the other by reducing its deleterious effects. Furthermore, our prior study of the Tsimane found that serum C-reactive protein (CRP), a general marker of inflammatory responses, was 60% higher in E3/E3 vs E4 carriers [46] , which is consistent with higher pathogen burden in E3 homozygotes.

Antagonistic pleiotropy, fluctuating selection, and heterozygotic advantage offer potential explanations for the worldwide prevalence of E4; there may be a fitness advantage to carrying E4 in some environments and/or at some life stages [23, 36] that trade-off against deleterious consequences [5, 9, 49]. While there may be some advantages (cognitive benefits and an increased ability to clear some infections) for E4 carriers during development when exposure risk to toxins [35] or disease is high [21, 23, 34], E4 is also associated with increased

cardiovascular and AD risk [4, 10], as well as decreased longevity after age 65 [49-51] in industrial populations. However, it is not known whether E4 affects late life mortality in populations with a high infectious burden.

Overall, our data do not support the antagonistic pleiotropy hypothesis with respect to early or late life advantages. E4 was generally beneficial to individuals facing a high parasite load regardless of age (H1). Findings among Tsimane children are consistent with the better cognitive performance of E4 carrying children in Mexico City [35] and Brazilian favelas [34]. Among Tsimane children nearly all measures of cognitive performance were positively associated with E4 status, although due to relatively small sample size, significance statistically significant difference was not detected. These gene-by-environment interactions, rather are consistent with balancing selection in areas with endemic parasitism, or fluctuating selection in environments with variable parasite exposure. Thus, E4 may be adaptive in some environments (with high parasite and pathogen load) and detrimental in others like industrialized populations with low parasite burden. Tropical regions rich in helminths have higher frequencies of the E4 allele [15], which may be protective against some parasites and infections [2, 22]. In many industrialized populations, the much higher prevalence of elevated cholesterol, blood pressure, and obesity make vascular dementias a significant concern; in these populations, E4 may negatively impact cognitive performance and dementia risk via increased amyloid deposits in the brain [8, 9]. Approximately 24% of Tsimane are carriers of E4, which is well within the range of what has been reported in Europe and North America, suggesting that our results are not due to outlier frequencies of APOE genotypes as have been reported in some populations [15, 46].

In populations facing a diversity of endemic pathogens, E4 may be protective against age-related cognitive declines, and possibly even AD. Meta-analyses conclude that bacterial pathogens also increase risk of AD, with spirochetal bacteria being associated with a 10-fold higher risk, and *Chlamydophila pneumonia* associated with a four-fold higher risk [52]. Moreover, the human amyloid-β peptide has been shown to have protective antimicrobial benefits against cerebral bacterial infections in transgenic mouse models [53]. More than two thirds of adult Tsimane suffer from intestinal helminths, and 50% of adults are anemic [37-39]. Thus in populations facing recurring pathogen exposure, the E4 allele may be adaptive by helping to clear infection and by decreasing the rate of cognitive decline during normal adult aging. In healthier populations, adults show progressive, slow attrition of cognitive processing and synapse loss [54], that is accelerated by E4 [7]. These adult aging processes may involve different functions of the APOE protein than are protective for the cognitive development of children [22, 34, 35]. In industrialized populations, sanitation, clean water, refrigeration, food safety, and medical advances have led to significant changes in the prevalence and incidence of parasitic and pathogenic infections, and thus may reduce the potential positive protective impacts of the E4 allele. In addition to vascular consequences of E4 that include higher risk of ischemic events, the potential benefit of the E4 allele for pathogen resistance may have less adaptive value for industrial populations with a lower pathogen burden from improved hygiene and modern medicine.

These results highlight the need for additional studies of E4 gene by environment interactions, especially in varied non-industrial environments, and for focusing on the neurobiology of middle age in addition to older ages and the need to take a life course perspective [54, 55]. Other studies find evidence of protective interactions between E4 alleles,

cognitive function, and a host of other phenotypic characteristics including testosterone [56], estrogen [57], and physical activity [58]. Considered together, the evidence suggests that the impacts of the E4 allele are not always deleterious, and may be adaptive and beneficial in some populations with a high infectious burden. Interestingly, we do not detect interactions between sex and E4 status in cognitive performance, e.g. the female-E4 excess of cognitive deficits in Alzheimer's disease [6], perhaps due to the younger ages of most subjects or to the lower levels of circulating testosterone reported in male Tsimane [44].

Limitations

While there is a strong association between eosinophil counts and chronic intestinal parasite load [41, 42], viral infections can also result in elevated eosinophil counts. That said, there was no interaction between E4 and other leucocyte subtypes (all $p>0.17$, see Table S5), or ESR ($p>0.2$), suggesting that current and past history of macro-parasites was driving this effect. Children in this study ranged in age from 6-18 years of age; while this period encompasses significant cognitive development, the critical window for brain growth may be before this age range [59].

Given the overall small sample size of our study and of the number of E4/E4 homozygous individuals, our study does not have the power to test for a heterozygote advantage. However, we consider this possibility to be highly likely, given the fact that none of the APOE alleles have gone to fixation among the Tsimane as well as the context-dependent advantages and disadvantages to the E4 allele. Other versions of antagonistic pleiotropy in which the benefits and costs of allelic variants vary environmentally could also explain the existing pattern of diversity. While our sample size is relatively small, our results are consistent with what has been reported in other South American populations with similar ancestry [21, 35].

Conclusions

While being a carrier of the ApoE4 allele is the single strongest risk factor of AD and cognitive decline in industrial populations, it is associated with greater cognitive performance in individuals facing a high parasite and pathogen load, suggesting that there may be advantages to the E4 allele in some environments. This may help explain the persistence and geographical distribution of such an otherwise deleterious allele.

Work Cited

1. Small, G.W., et al., *Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease.* Proceedings of the National Academy of Sciences, 2000. **97**(11): p. 6037-6042.
2. Azevedo, O.G.R., et al., *Apolipoprotein E Plays a Key Role against Cryptosporidial Infection in Transgenic Undernourished Mice.* PLoS One, 2014. **9**(2): p. e89562.
3. van Exel, E., et al., *VAscular factors and markers of inflammation in offspring with a parental history of late-onset alzheimer disease.* Archives of General Psychiatry, 2009. **66**(11): p. 1263-1270.
4. Alzheimer's, A., *2016 Alzheimer's disease facts and figures.* Alzheimer's & Dementia, 2016. **12**(4): p. 459-509.
5. Altmann, A., et al., *Sex modifies the APOE-related risk of developing Alzheimer disease.* Ann Neurol, 2014. **75**(4): p. 563-73.
6. Cacciottolo, M., et al., *The APOE4 allele shows opposite sex bias in microbleeds and Alzheimer's disease of humans and mice.* Neurobiology of aging, 2016. **37**: p. 47-57.
7. Weiner, M.W., et al., *2014 Update of the Alzheimer's Disease Neuroimaging Initiative: A review of papers published since its inception.* Alzheimers Dement, 2015. **11**(6): p. e1-120.
8. Mahley, R.W. and S.C. Rall Jr, *Apolipoprotein E: far more than a lipid transport protein.* Annual review of genomics and human genetics, 2000. **1**(1): p. 507-537.
9. Hagberg, J.M., K.R. Wilund, and R.E. Ferrell, *APO E gene and gene-environment effects on plasma lipoprotein-lipid levels.* Physiological Genomics, 2000. **4**(2): p. 101-108.
10. Eichner, J.E., et al., *Apolipoprotein E Polymorphism and Cardiovascular Disease: A HuGE Review.* American Journal of Epidemiology, 2002. **155**(6): p. 487-495.
11. Song, Y., M.J. Stampfer, and S. Liu, *Meta-Analysis: Apolipoprotein E Genotypes and Risk for Coronary Heart Disease.* Annals of Internal Medicine, 2004. **141**(2): p. 137-147.
12. Bennet, A.M., et al., *ASsociation of apolipoprotein e genotypes with lipid levels and coronary risk.* JAMA, 2007. **298**(11): p. 1300-1311.
13. Go, A.S., et al., *Executive Summary: Heart Disease and Stroke Statistics: 2013 Update: A Report From the American Heart Association.* Circulation, 2013. **127**(1): p. 143-146.
14. Prince, M., et al., *The global prevalence of dementia: A systematic review and metaanalysis.* Alzheimer's & Dementia, 2013. **9**(1): p. 63-75.e2.
15. Eisenberg, D.T.A., C.W. Kuzawa, and M.G. Hayes, *Worldwide allele frequencies of the human apolipoprotein E gene: Climate, local adaptations, and evolutionary history.* American Journal of Physical Anthropology, 2010. **143**(1): p. 100-111.
16. Finch, C.E. and C.B. Stanford, *Meat-adaptive genes and the evolution of slower aging in humans.* The Quarterly Review of Biology, 2004. **79**(1): p. 3-50.
17. Fullerton, S.M., et al., *Apolipoprotein E variation at the sequence haplotype level: implications for the origin and maintenance of a major human polymorphism.* The American Journal of Human Genetics, 2000. **67**(4): p. 881-900.
18. Singh, P.P., M. Singh, and S.S. Mastana, *APOE distribution in world populations with new data from India and the UK.* Annals of Human Biology, 2006. **33**(3): p. 279-308.
19. Ghasemian, R.F., S. Borinskaya, and N. Yankovsky, *Worldwide Distribution of Apolipoprotein E Gene Alleles: is APOE* e4 Allele a Factor of Adaptation to Climate in Humans?* 2005.
20. Mueller, T., et al., *Apolipoprotein E (APOE) allele frequencies in chronic and self-limited hepatitis C suggest a protective effect of APOE4 in the course of HCV infection.* Liver International, 2016: p. n/a-n/a.

21. Oria, R.B., et al., *APOE4 protects the cognitive development in children with heavy diarrhea burdens in Northeast Brazil.* Pediatric research, 2005. **57**(2): p. 310-316.
22. Oria, R., et al., *ApoE polymorphisms and diarrheal outcomes in Brazilian shanty town children.* Brazilian Journal of Medical and Biological Research, 2010. **43**(3): p. 249-256.
23. Oriá, R.B., et al., *Role of apolipoprotein E4 in protecting children against early childhood diarrhea outcomes and implications for later development.* Medical Hypotheses, 2007. **68**(5): p. 1099-1107.
24. Gale, S.D., et al., *Infectious disease burden and cognitive function in young to middle-aged adults.* Brain, Behavior, and Immunity, 2016. **52**: p. 161-168.
25. Gale, S.D., et al., *Interaction between <italic>Helicobacter pylori</italic> and Latent Toxoplasmosis and Demographic Variables on Cognitive Function in Young to Middle-Aged Adults.* PLoS One, 2015. **10**(1): p. e0116874.
26. Katan, M., et al., *Infectious burden and cognitive function The Northern Manhattan Study.* Neurology, 2013. **80**(13): p. 1209-1215.
27. Hall, A., et al., *A review and meta-analysis of the impact of intestinal worms on child growth and nutrition.* Matern Child Nutr, 2008. **4 Suppl 1**: p. 118-236.
28. Bowles, S., H. Gintis, and M. Osborne, *Incentive-enhancing preferences: Personality, behavior, and earnings.* The American Economic Review, 2001. **91**(2): p. 155-158.
29. Strenze, T., *Intelligence and socioeconomic success: A meta-analytic review of longitudinal research.* Intelligence, 2007. **35**(5): p. 401-426.
30. Kaplan, H.S., P.L. Hooper, and M. Gurven, *The evolutionary and ecological roots of human social organization.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2009. **364**(1533): p. 3289-3299.
31. Gurven, M., H. Kaplan, and M. Gutierrez, *How long does it take to become a proficient hunter? Implications for the evolution of extended development and long life span.* J Hum Evol, 2006. **51**(5): p. 454-70.
32. Hooper, P.L., et al., *Inclusive fitness and differential productivity across the life course determine intergenerational transfers in a small-scale human society.* Proceedings of the Royal Society of London B: Biological Sciences, 2015. **282**(1803): p. 20142808.
33. Hawkes, K., et al., *Grandmothering, menopause, and the evolution of human life histories.* Proceedings of the National Academy of Sciences, 1998. **95**(3): p. 1336-1339.
34. Mitter, S.S., et al., *Apolipoprotein E4 influences growth and cognitive responses to micronutrient supplementation in shantytown children from northeast Brazil.* Clinics, 2012. **67**(1): p. 11-18.
35. Wright, R.O., et al., *Apolipoprotein E genotype predicts 24-month bayley scales infant development score.* Pediatric research, 2003. **54**(6): p. 819-825.
36. Williams, G.C., *Pleiotropy, Natural Selection, and the Evolution of Senescence.* Evolution, 1957. **11**: p. 398-411.
37. Gurven, M., et al., *Inflammation and infection do not promote arterial aging and cardiovascular disease among lean Tsimane forager-horticulturalists.* PLoS ONE, 2009. **4**(8): p. e6590.
38. Blackwell, A., et al., *Immune Function in Amazonian Horticulturalists.* Ann Hum Biol, 2016. **43**: p. 382–396.
39. Blackwell, A.D., et al., *Helminth infection, fecundity, and age of first pregnancy in women.* Science, 2015. **350**(6263): p. 970-972.
40. Gurven, M., H. Kaplan, and A. Zelada Supa, *Mortality experience of Tsimane Amerindians: regional variation and temporal trends.* American Journal of Human Biology, 2007. **19**: p. 376-398.
41. O'Connell, E.M. and T.B. Nutman, *Eosinophilia in Infectious Diseases.* Immunology and allergy clinics of North America, 2015. **35**(3): p. 493-522.

42. Nair, M.G. and D.B.R. Herbert, *Immune polarization by hookworms: taking cues from T helper type 2, type 2 innate lymphoid cells and alternatively activated macrophages.* Immunology, 2016. **148**(2): p. 115-124.
43. Rocha, M., et al., *Pathogenetic factors of acute schistosomiasis mansoni: correlation of worm burden, IgE, blood eosinophilia and intensity of clinical manifestations.* Tropical Medicine & International Health, 1996. **1**(2): p. 213-220.
44. Trumble, B.C., et al., *Testosterone and male cognitive performance in Tsimane forager-horticulturalists.* American Journal of Human Biology, 2014: p. n/a-n/a.
45. Gurven, M., et al., *Cognitive performance across the life course among Bolivian forager-farmers with limited schooling.* Developmental Psychology, 2016.
46. Vasunilashorn, S., et al., *Inflammatory gene variants in the Tsimane, an indigenous Bolivian population with a high infectious load.* Biodemography and Social Biology, 2011. **57**(1): p. 33-52.
47. Prieto, R. and J.E. Richter, *Eosinophilic esophagitis in adults: an update on medical management.* Current gastroenterology reports, 2013. **15**(6): p. 1-9.
48. Williamson, L.L., et al., *Got worms? Perinatal exposure to helminths prevents persistent immune sensitization and cognitive dysfunction induced by early-life infection.* Brain, behavior, and immunity, 2016. **51**: p. 14-28.
49. Ewbank, D.C., *The APOE gene and differences in life expectancy in Europe.* J Gerontol A Biol Sci Med Sci, 2004. **59**(1): p. 16-20.
50. Kulminski, A.M., et al., *Age, Gender, and Cancer but Not Neurodegenerative and Cardiovascular Diseases Strongly Modulate Systemic Effect of the Apolipoprotein E4 Allele on Lifespan.* PLoS Genet, 2014. **10**(1): p. e1004141.
51. Soerensen, M., et al., *Evidence from case–control and longitudinal studies supports associations of genetic variation in APOE, CETP, and IL6 with human longevity.* AGE, 2012. **35**(2): p. 487-500.
52. Maheshwari, P. and G.D. Eslick, *Bacterial infection and Alzheimer's disease: a meta-analysis.* Journal of Alzheimer's disease: JAD, 2015. **43**(3): p. 957-966.
53. Kumar, D.K.V., et al., *Amyloid-β peptide protects against microbial infection in mouse and worm models of Alzheimer's disease.* Science translational medicine, 2016. **8**(340): p. 340ra72-340ra72.
54. Finch, C.E., *The neurobiology of middle-age has arrived.* Neurobiology of aging, 2009. **30**(4): p. 515-520.
55. Finch, C.E. and G. Ruvkun, *The genetics of aging.* Annual review of genomics and human genetics, 2001. **2**(1): p. 435-462.
56. Panizzon, M.S., et al., *Interaction of APOE genotype and testosterone on episodic memory in middle-aged men.* Neurobiology of aging, 2014. **35**(7): p. 1778. e1-1778. e8.
57. Yaffe, K., et al., *Estrogen use, APOE, and cognitive decline Evidence of gene–environment interaction.* Neurology, 2000. **54**(10): p. 1949-1954.
58. Nichol, K., et al., *Exercise improves cognition and hippocampal plasticity in APOE ε4 mice.* Alzheimer's & Dementia, 2009. **5**(4): p. 287-294.
59. Kuzawa, C.W., et al., *Metabolic costs and evolutionary implications of human brain development.* Proceedings of the National Academy of Sciences, 2014. **111**(36): p. 13010-13015.

Figure Captions

Figure 1: Predicted Z Scores for Composite Fluid Cognitive Performance (n=242 adults aged 30+), APOE4 Status, and four levels of Eosinophil count, controlling for age, sex, education, and Spanish speaking ability with community ID as a random effect. Following [41], categories were broken into no eosinophilia <500 eosinophils/uL, mild eosinophilia <1500/uL, marked eosinophilia <2500/uL, and very marked eosinophilia ≥2500/uL.

Table 1: Mixed effects linear regressions examining associations between APOE4, eosinophil count (eosinophils/uL x1000), and cognitive performance Z scores for fluid cognition tasks (n=242 adults aged 30+), see Table S1 for full results. Village ID is included as a random effect (not shown).

Figure 2: Graphs of each cognitive measure by eosinophil count for carriers of APOE4 (dashed line) and ApoE3 (solid) for N=242 adults aged 30-88. Note that for nearly all measures of cognitive function E3/E3 genotypes perform better with non-clinical eosinophil counts (eosinophil count <500 cells/uL) similar to what is seen in industrial populations, while E4 carriers perform better in participants with higher eosinophil counts.

Figure 3: Graphs of each cognitive measure by eosinophil count for carriers of APOE4 (dashed line) and ApoE3 (solid) for N=138 post-reproductive adults aged 45-88.

Figure S1: STROBE flow chart depicting sampling

Figure S2 Graphs of each cognitive measure by eosinophil count for carriers of APOE4 (dashed line) and ApoE3 (solid) for N=124 children aged 6-18.

Table S1: Mixed effects linear regressions examining associations between APOE4, eosinophil count, and cognitive performance Z scores (n=242 adults aged 30+). Village ID is also included as a random effect (not shown).

Table S2: Mixed effects linear regressions examining associations between APOE4, eosinophil count, and cognitive performance Z scores (n=124 children < age 18). Village ID is also included as a random effect (not shown). Note anthropometric measurements were only available for n=85 children.

Table S3: Mixed effects linear regressions examining associations between APOE4, eosinophil count, and cognitive performance Z scores (n=124 children < age 18). Village ID is also included as a random effect (not shown). Note anthropometric measurements were only available for n=85 children.

Table S4: Mixed effects linear regressions examining associations between ApoE4, eosinophil count, and cognitive performance Z scores (n=138 adults aged 45+). Village ID is also included as a random effect (not shown).

Table S5: Mixed effects linear regressions examining associations between APOE4, four leucocyte subtypes, composite fluid cognitive performance Z scores (n=242 adults aged 30+). Village ID is also included as a random effect (not shown).

Table 1: Mixed effects linear regressions examining associations between APOE4, eosinophil count (eosinophils/uL x1000), and cognitive performance Z scores for fluid cognition tasks (n=242 adults aged 30+), see Table S1 for full results. Village ID is included as a random effect (not shown).

	Short Term Recall		**Long Term Recall**		**Composite Digit Forward**		**Total Composite Fluid**	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
APOE4	-0.018	-0.666**	-0.069	-0.656*	-0.089	-0.525□	-0.137	-0.760**
E4 x Eosin Count	-	0.584***	-	0.517*	-	0.415*	-	0.555**
Age	-0.030***	-0.030***	-0.030***	-0.029***	-0.013**	-0.015**	-0.024***	-0.024***
Sex	0.070	0.032	-0.111	-0.148	0.628***	0.608***	0.476***	0.438***
Education	0.086	0.091	0.012	0.016	0.147	0.189	0.138	0.149
Spanish	-0.042	-0.047	0.169***	0.163***	0.047	0.046	0.079	0.076
Eosinophil Count	0.067	-0.087	0.013	-0.122	-0.015	-0.115	0.005	-0.148
AIC	592.9	584.5	665.0	661.1	656.1	655.2	632.6	627.0
Pseudo R2	0.199	0.234	0.180	0.200	0.174	0.184	0.213	0.240

□=p<0.1, *P≤0.05, ** P≤0.01, ** P≤0.001

Figure 1

Click here to download Figure Figure1.tif

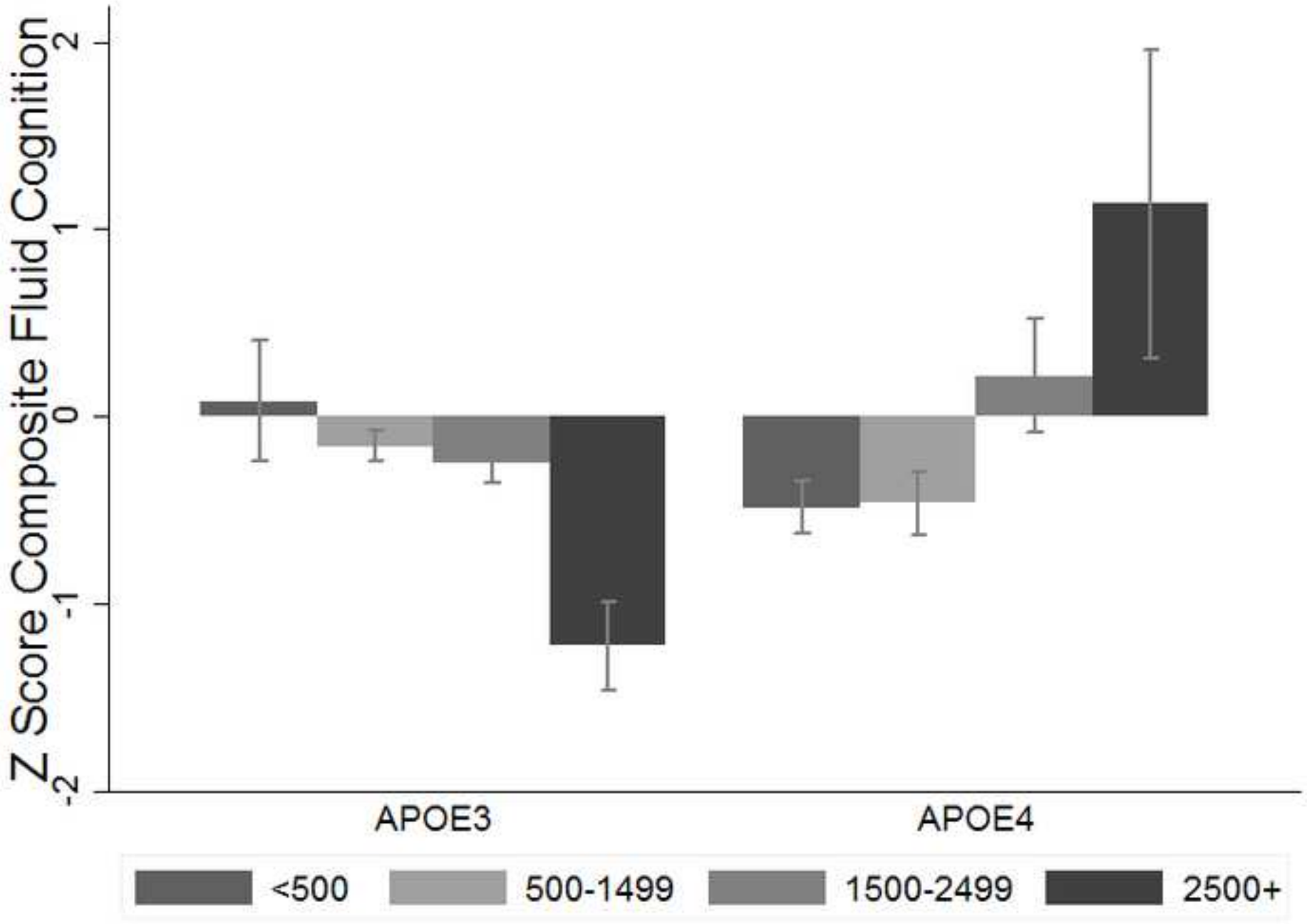


Figure 2

Click here to download Figure Figure2.tif

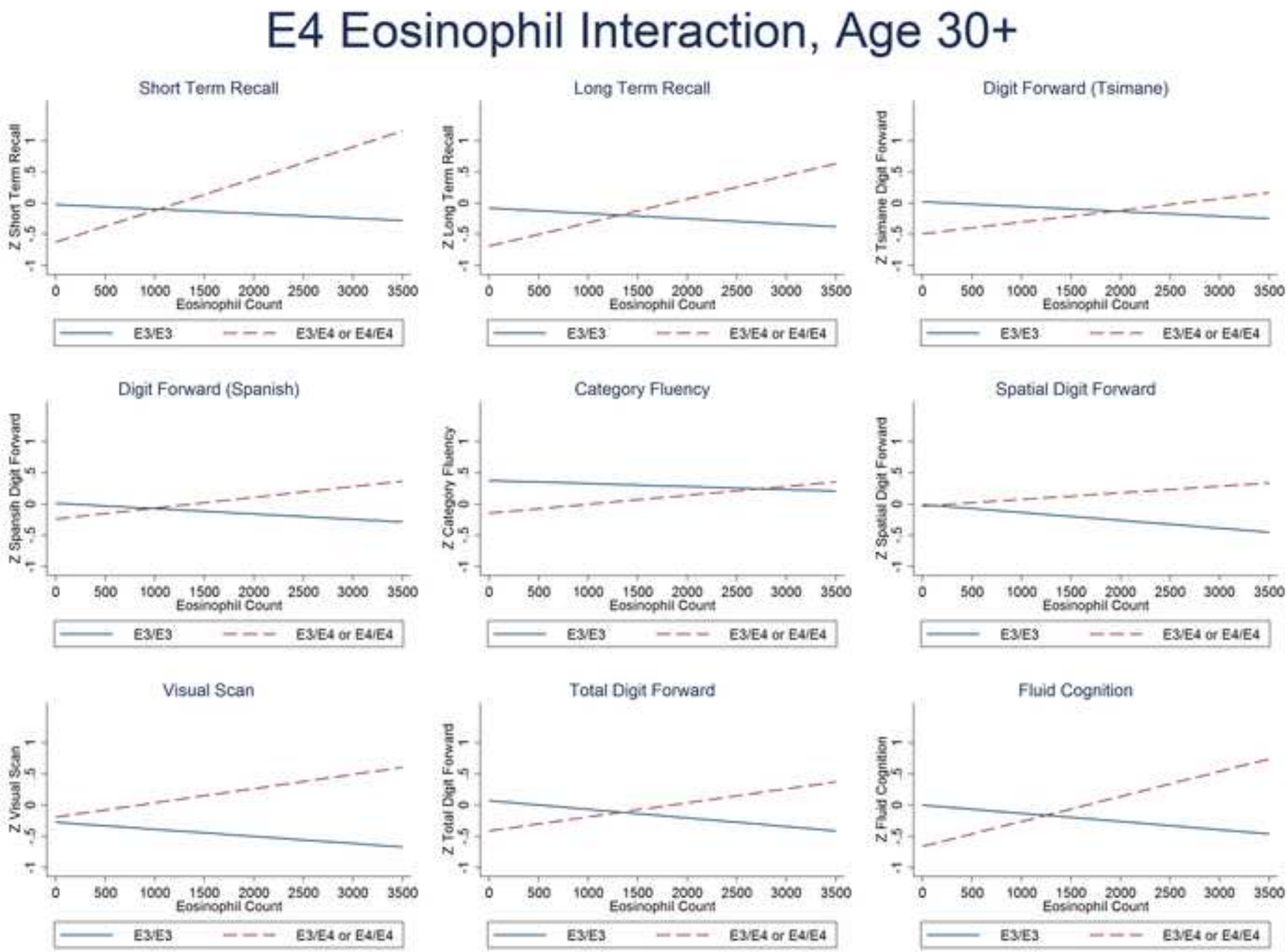
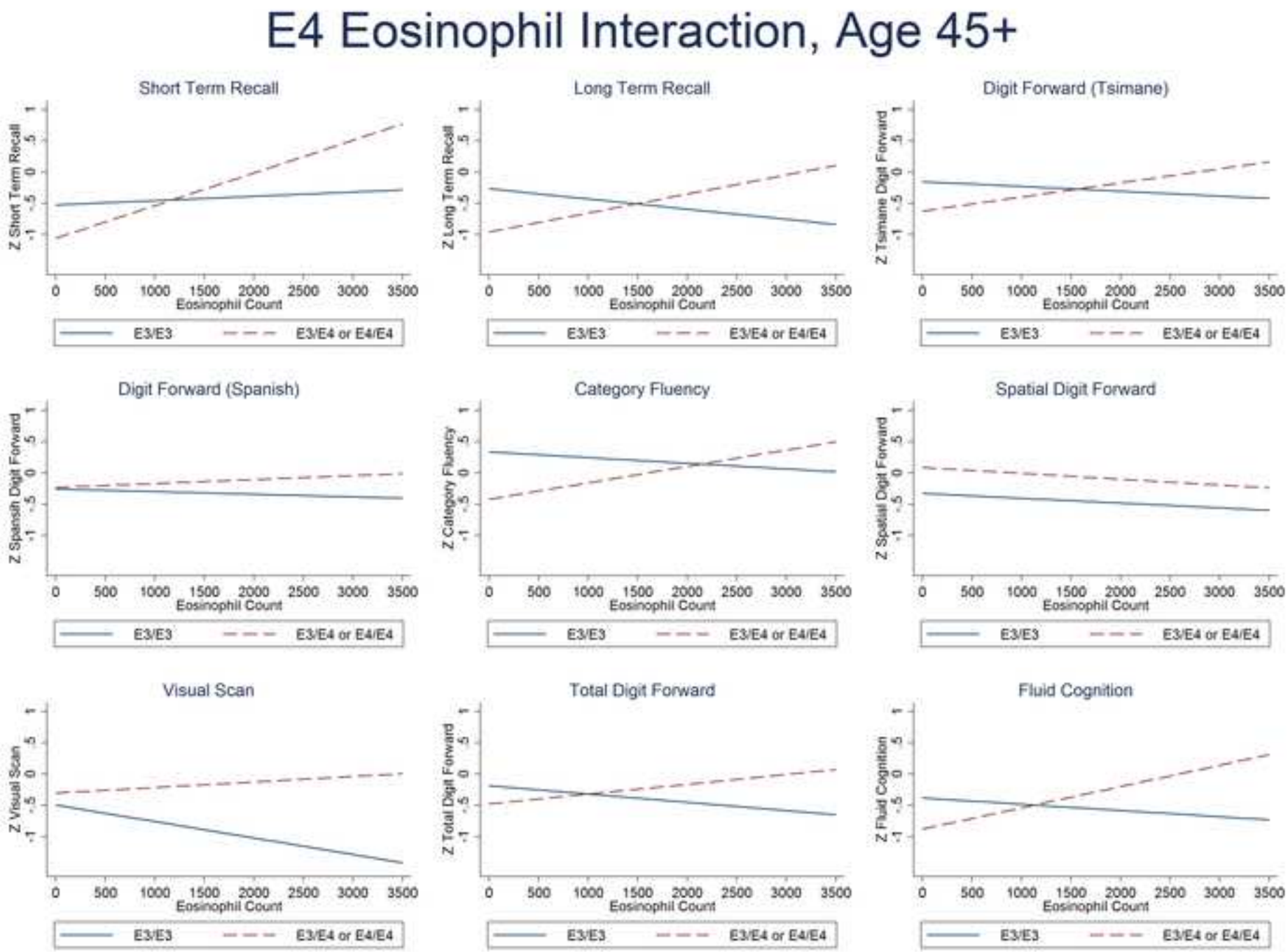


Figure 3

Click here to download Figure Figure3.tif



S1 Fig S1


Click here to access/download
Supporting Information
FigureS1.tif

S2 Fig S2


Click here to access/download
Supporting Information
FigureS2.tif

S1 Table S1

S3 Table S3

S5 Table S5