APOE ALLELE FREQUENCIES AND REGIONAL RICHNESS OF INTRACELLULAR PATHOGENS AND EXTRACELLULAR PATHOGENS

by

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ABSTRACT

Research into the genetic factors that contribute to human health and longevity has found that individual's apolipoprotein E (APOE) genotype is linked to their risk for developing Alzheimer's disease and cardiovascular diseases. Despite its negative impact on health and longevity, the E4 allele is nonetheless maintained by selection in various frequencies worldwide. I examined which APOE allele frequencies are higher in populations living in regions rich in intracellular versus extracellular pathogens. Consistent with hypothesis that the E4 allele promotes inflammation and may boost antiviral immune defenses, I predicted that populations inhabiting regions high in intracellular pathogen richness would exhibit higher frequencies of E4 alleles than those living in regions with lower numbers of intracellular pathogens. Neither intracellular nor extracellular pathogen richness predicted E2 allele frequency, higher extracellular pathogen richness predicted greater E3 allele frequency, and lower extracellular pathogen richness and higher intracellular pathogen richness predicted greater E4 allele frequency. The results of the current research corroborate previous findings suggesting that the E4 allele provides immunological benefits, particularly in regards to antiviral immunity and suggests that an intracellular rich environment may impose a selection pressure that increases the frequency of this allele.

Introduction

Research into the genetic factors that contribute to human health and longevity has found that individual's apolipoprotein E (APOE) genotype is linked to their risk for developing Alzheimer's disease and cardiovascular diseases. Of the polymorphisms, the APOE4 (E4) allele increases the risk for developing these disorders. For example, approximately 50% of individuals diagnosed with Alzheimer's disease possess at least one E4 allele (Raber et al., 2004; Saunders et al. 1993). Despite its negative impact on health and longevity, the E4 allele is nonetheless maintained by selection in various frequencies across human populations, with the highest frequencies found in indigenous populations (Abondio et al., 2019). Accordingly, some researchers have hypothesized that possessing the E4 alleles confers fitness benefits, particularly for populations whose lifestyles resemble those of humans' ancestral past. In the current research, I investigated the possibility that the E4 allele is maintained in populations because it bolsters immunological defenses against certain classes of infectious diseases. Specifically, I examined whether E4 allele frequency is higher in populations living in regions rich in intracellular versus extracellular pathogens.

The APOE gene is involved in many physiological functions involving lipids (Frieden and Garai, 2013). Approximately 60% of the ApoE protein is synthesized by the liver, and the glial cells and macrophages produce the next highest levels (Basu et al., 1982; Newman et al., 1985). The C-terminus of the protein is primarily bound to lipids to form lipoproteins such as chylomicrons, very low-density lipoproteins (VLDL), and high-density lipoproteins (HDL). The N-terminus has high affinity for the LDL receptor family as well as glycosaminoglycans, both for lipoprotein reuptake (Strickland et al., 2002; Mahley et al., 1999), allowing the protein to be used for aiding in cholesterols entering the cells. In addition to influencing the function of lipids

in the periphery, the ApoE protein is also involved in regulating neuroplasticity (Lagercrantz and Ringstedt, 2001) and synaptogenesis (Mauch et al., 2001). Research suggests that transport or reuptake of cholesterol is involved in the development and plasticity of neural circuitry, and that apoE produced by glial cells plays a role in synaptogenesis (Lagercrantz and Ringstedt, 2001; Mauch et al., 2001).

The APOE allele has three known haplotypes due to a combination of two mutations at positions 112 and 158 resulting in the E2, E3, and E4 proteins. ApoE2 has a cystine residue at both 112 and 158, ApoE3 has a cystine at 112 and an arginine at 158, and ApoE4 has an arginine at both 112 and 158 (Weisgraber et al., 1981). ApoE2 has reduced LDLR affinity and reduced ability to bind to the receptor (Weisgraber et al, 1982). ApoE4 has a higher affinity to the LDL receptor, which leads to the downregulation of LDL receptors and higher LDL levels in the blood (Malloy et al., 2004). The Arg mutation on the ApoE4 protein causes the protein to only partially fold in the hinge region of the protein, resulting in ApoE4 being flagged more often as misfolded, especially in the brain (Elliott et al., 2011). The recognition of the misfolded E4 protein can lead to higher inflammation due to stimulating NF-kB (Cao, 2014; Chaudhari, 2014).

Decades of research have demonstrated that the E4 allele (compared to the other alleles) is strongly linked to the development of Alzheimer's disease and cardiovascular diseases (Spinney, 2014). Despite its negative effects on health, however, the E4 allele remains in virtually every human population studied (Abondio, 2019) with some populations having much higher frequencies than others. Accordingly, researchers have hypothesized that there may be a selection pressure driving the maintenance of this allele at appreciable frequencies. Research finds, for example, that E4 allele frequency is highest near the equator and the poles (Eisenberg et al., 2010). Given that the APOE gene is primarily involved in cholesterol metabolism, the

metabolic rate necessary for an environment could select for an APOE allele. Specifically, higher metabolic rates are necessary in extremely hot environments like those around the equator and extremely cold environments like those around the poles which suggests that the E4 allele is more fit for high metabolic needs (Eisenberg et al, 2010). Consistent with this hypothesis, indigenous populations in Africa and Oceania have high metabolic demand in their climate and are populations where the allele frequency is highest (Singh et al., 2006).

Beyond the positive metabolic effects of the E4 allele, some research suggests that it may also provide a protective advantage against infectious diseases. In particular, the E4 allele may lend additional protection against viruses such as Hepatitis C (HCV) and certain parasites such as *Giardia* and cryptosporidium. For example, (HCV) circulates in blood in LDL and HDL lipoproteins, and infects a cell primarily through LDLr. The lowered rates of LDLr in individuals with E4 and the competing high levels of non-HCV infected LDL in the blood makes it difficult for virus to infect liver cells. (Fabris et al., 2005; Wozniak, 2002). Others find that in nonindustrial areas of the world with high rates of childhood diarrhea due to enteric parasites that lead to chronic malnutrition, possessing an E4 allele protects cognitive development, presumably via enhanced cholesterol absorption from the diet and higher blood levels of LDL (Lorntz, 2006; Oriá et al., 2007). ApoE4 might also be involved in preventing children from susceptibility to enteric parasites. Still others find that in adults exposed to certain types of parasites, those who have an E4 allele (compared to those who do not) exhibit lower levels of C-reactive protein, a marker for systematic inflammation (Vasunilashorn et al., 2011).

These findings suggest that having an E4 allele may be protective against both intracellular pathogens, like viruses, and large, extracellular pathogens, like helminths. However, separate research suggests that the changes in lipid metabolism and immune function found in

those possessing an E4 allele (compared to those without an E4 allele) may be especially beneficial for host defenses against intracellular pathogens. Extracellular pathogens, such as larger helminth parasites, elicit qualitatively different immune responses and exhibit different pathogenic mechanisms than intracellular pathogens, such as viruses. Large extracellular pathogens primarily elicit the T_H2-type response, which involves activation of T_H2 cells and increases in T_H2 cytokines such as IL-4, IL-13, IL-21, and IL-25, all which downregulate the T_H1 type response that is involved in intracellular infections such as INF-γ (Rutitzky et al., 2007). Intracellular pathogens, on the other hand, first activate the innate immune response when pattern recognition receptors (PPRs) such as toll-like receptors (TLR) recognize pathogen-associated molecular patterns (PAMPs) (Ishii et al., 2008). PPRs then activate 3 signaling pathways: mitogen-activated protein kinases (MAPKs), interferon regulator factors (IRFs), and nuclear factor (NF)-κB (Rasmussen et al., 2007).

While not directly involved in the immune system, the variation in the ApoE protein is linked to pathogen susceptibility by activating general changes in immune function, preventing pathogens from binding to lipoproteins, or preventing the use of apoE receptors (Finch & Morgan, 2007). ApoE is mainly expressed in liver cells, but is also expressed in macrophages and glial cells, so variations in isoform can promote changes in the activity of these cell types. Research finds that those with the E4 allele have increased levels of IL-8 and TNF-α than those with the E3 and E2 alleles (Drabe et al. 2001) suggesting it is a pro-inflammatory phenotype (Trotter et al., 2011). TNF-a, in particular, promotes inflammation and initiates antiviral responses (Abreu-Martin et al., 1995; Bradham et al., 1998). Given that the E4 allele is associated with heightened and elevated levels of signaling proteins involved in antiviral

immunity (Dobson et al., 2006) possessing an E4 allele may be especially protective against intracellular versus extracellular pathogens.

With these insights in mind, I sought to extend previous work by examining relationships between regional variability in richness of different classes of pathogens and APOE allele frequency. I aimed to examine whether richness of intracellular versus extracellular pathogens predicted E4 allele frequency, consistent with previous research suggesting that this allele is maintained in populations because it confers immunological benefits (Fabris et al, 2005; Trumble et al., 2006; Wozniak, 2002). Specifically, I predicted that E4 allele frequency would be especially high in regions rich in intracellular pathogens, given the association between this allele and elevated inflammation, a critical component of the immune system's response to viruses and intracellular bacteria (Ophir et al., 2005).

Method

Procedure and Materials

In order to examine the relationship between regional pathogen richness and APOE allele frequency, I accessed previously published data on (a) estimated frequency of the three most common APOE alleles/isoforms (ε2, ε3, ε4) in 265 populations worldwide spanning every continent except Antarctica, (b) estimated richness of intracellular (i.e., viruses) and extracellular (i.e., helminths) pathogens in each of the countries where these populations reside, and (c) a number of country-level variables that may confound the relationship between APOE allele frequency and richness of these infectious diseases (e.g., average temperature, latitude, rainfall, etc.).

Country-level richness of intracellular and extracellular pathogens. Estimates of intracellular and extracellular pathogen richness in 224 countries were extracted from the Global

Infectious Disease and Epidemiology Network (GIDEON) database using a presence/absence matrix. Following previously published procedures (Fumagalli et al., 2010; Manczinger et al., 2019; Prugnolle et al., 2005). The GIDEON database provides up-to-date information about the presence of disease-causing pathogens worldwide, and data extracted from this network has been used to examine how pathogen richness shapes genetic diversity in several previous studies (Fumagalli et al., 2010; Manczinger et al., 2019; Prugnolle et al., 2005). Only species naturally transmitted in each country were included, such that species recently entering the country due to tourism or immigration were excluded. Species eradicated by recent vaccination programs were included given that our objective was to examine whether historical pathogen richness in each region shaped APOE allele frequency (i.e., over thousands of years), not contemporary pathogen richness (i.e., which is unlikely to impose as strong of a selection pressure as historical richness).

As a measure of intracellular pathogen richness, I summed the number of unique disease-causing viruses present in each country. I chose to focus specifically on viruses because these microorganisms are unambiguously obligate intracellular pathogens, whereas many bacteria and protozoan parasites have both intracellular and extracellular stages during infection (Fumagalli et al., 2010; Girardin et al., 2002; Manczinger et al., 2019; Prugnolle et al., 2005). As a measure of extracellular pathogen richness, I summed the number of unique disease-causing helminths in each country. Helminths are large parasitic worms, including trematodes, cestodes, and nematodes. While larval stages of some helminths may have intracellular components, helminth infections are primarily extracellular in nature, and elicit immune responses that limit tissue damage from these large pathogenic organisms (Fumagalli et al., 2010; Girardin et al., 2002; Maizels et al., 2016). Higher numbers of unique viruses and helminths represented higher levels of intracellular and extracellular pathogen richness, respectively.

APOE allele frequency and covariates. Data from the frequency of APOE ε2, ε3, ε4 alleles were accessed from a previous publication (Eisenberg et al., 2010). These data were aggregated from various sources, including a previously published paper (Singh et al., 2006) and the Human Genome Diversity Cell Line Panel (Cann et al., 2002). In these data, elderly and clinical populations were excluded based on previous research suggesting a survival bias in these populations (Eisenberg et al., 2010). Allele frequencies were reflected as a percentage of each population that inherited each allele.

In addition to the genetic data, I also included the same covariates tested by Eisenberg and colleagues (2010) in my analysis. These included the latitude and longitude of each country, elevation, average rainfall, average temperature, and whether the country was in the northern or southern hemisphere. These data were extracted from previously published resources, including the GlobalTempSim program (Spokas, 2007) and the USGS Seamless Elevation dataset (2008).

Data Analysis Plan

I analyzed my data using linear mixed-effects models fit using R online statistical software version 3.6.0 (R Core Team, 2019), with lme4 version 1.1-2.1 (Bates et al., 2015), and lmerTest version 3.1-0 (Kuznetsova e al., 2017). To control for the hierarchical organization of the data with countries nested within continents, which were themselves nested within supercontinents, I included random effects of country, continent, and supercontinent in each model. Using multiple regression models, I tested whether intracellular or extracellular pathogens predicted frequency of each allele, controlling for latitude/longitude, average rainfall, average temperature, elevation, and hemisphere (per Eisenberg, 2010).

Results

Descriptive statistics are shown in Table 1. For the ε2 allele, results revealed that only elevation significantly predicted its frequency. Specifically, higher elevation predicted lower frequencies of this allele, b = -.001, SE = -.0006, t = -2.78, p = .006. No other variable significantly predicted frequency of this allele (ps > .19).

For the ε3 allele, results revealed that higher frequency was predicted by higher elevation, b = .003, SE = .001, t = 2.47, p = .02, and less rainfall, b = -1.32, SE = .58, t = -2.26, p = .03. Additionally, higher ε3 frequency was also predicted by greater extracellular pathogen richness, b = .51, SE = .20, t = 2.54, p = .01, but not intracellular pathogen richness (p = .20). No other predictors reached significant (ps > .11).

For the ε4 allele, greater frequency was found at higher latitudes, b = .18, SE = .07, t = -2.58, p = .01, and regions with greater rainfall, b = 1.44, SE = .35, t = 4.12, p < .001. Further, greater frequency of this allele was associated with greater intracellular pathogen richness, b = .60, SE = .13, t = 4.44, p < .001, but lower extracellular pathogen richness, b = -.27, SE = .10, t = -2.69, p = .008 (see Figures 1 and 2). No other predictors reached significance (ps > .33).

Discussion

Given the proposed link between APOE genotype and immune function (Fabris et al, 2005; Trumble et al., 2006; Wozniak, 2002), I sought to investigate relationships between APOE allele frequency worldwide and regional richness of intracellular pathogens and extracellular pathogens. Consistent with hypothesis that the E4 allele promotes inflammation and may boost antiviral immune defenses (Fabris et al, 2005; Ophir et al., 2005; Trumble et al., 2006; Wozniak, 2002). I predicted that populations inhabiting regions high in intracellular pathogen richness would exhibit higher frequencies of E4 alleles than those living in regions with lower numbers of intracellular pathogens.

My results revealed that in regard to location, the E2 allele was only predicted by elevation such that lower elevations predicted the E2 allele. The E3 allele was predicted by higher elevation and less rainfall. The E4 allele was predicted by higher latitudes and greater rainfall. In regard to pathogen richness, neither intracellular nor extracellular pathogen richness predicted E2 allele frequency, higher extracellular pathogen richness predicted greater E3 allele frequency, and lower extracellular pathogen richness and higher intracellular pathogen richness predicted greater E4 allele frequency.

The results of the current research corroborate previous findings suggesting that the E4 allele provides immunological benefits, particularly in regards to antiviral immunity (Dobson et al., 2006) and suggests that an intracellular rich environment may impose a selection pressure that increases the frequency of this allele. Since the frequency of the E4 allele was also negatively related to extracellular pathogen richness, it provides evidence that possessing this allele may be detrimental to the immune response to helminths and other large parasites. Frequency of the E3 allele, on the other hand, was predicted by higher extracellular pathogen research, suggesting an involvement with the E3 allele and extracellular pathogen defense.

Research into qualitative differences between the immune response to intracellular versus extracellular pathogens may lend insight into why the E4 allele is both more common in regions with high intracellular pathogen richness and less common in regions with high extracellular pathogen richness. The immune response to intracellular pathogens typically involves the activation of the MAP kinase signaling pathway, interferon regulator factors (IRFs), and NFκB (Rasmussen et al., 2007) leading to increased levels of IL-8 and TNF-α, which promote inflammation (Abreu-Martin et al., 1995). The immune response to extracellular pathogens, on

the other hand, activates T_H2 cells which increases cytokines that downregulate the T_H1 type response of intracellular infections (Rutitzky et al., 2007).

The E4 allele is linked to higher TNF-α, and IL-8 levels (Drabe et al. 2001) and elevated NF-κB activation (Ophir et al., 2005) suggesting it is involved in the proinflammatory response to intracellular pathogens. This suggests E4 could provide a benefit against viral illnesses. Since the extracellular pathway does not elicit the same proinflammatory response, E4 may not provide a benefit against large parasites such as helminth-related infections.

Limitations of this research involve the estimation of pathogen richness. There are other pathogens, such as bacteria and protozoan parasites, that were excluded from my estimation of intracellular and extracellular pathogen richness due to their inflectious pathways involving both intracellular and extracellular stages. Also, this research is cross-sectional and is associative. Accordingly, laboratory studies need to be conducted to determine how the APOE genotype influences immune function. For example, Jofre-Monseny (2008) conducted a review comparing APOE genotypes and inflammatory markers to analyze which markers were higher for different genotypes and found a positive correlation between individuals with the E4 allele and inflammatory markers such as TNF-α and NFκB.

The current research found that higher regional richness of intracellular pathogens is associated with greater frequencies of E4 alleles, suggesting that the E4 allele may confer some benefit in the immune response to intracellular pathogens. These results lay the groundwork for future research to be done to examine the exact benefit the E4 allele provides to immune system health and why it is an allele that remains selected for in specific populations.

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Table 1

Descriptive Statistics

Variable	*M*(*SD*)
Intracellular Pathogen Richness	45.34 (4.50)
Extracellular Pathogen Richness	24.17 (5.18)
E2 Frequency (%)	8.34 (6.20)
E3 Frequency (%)	75.49 (10.38)
E4 Frequency (%)	16.00 (8.09)

Note. Intracellular and extracellular pathogen richness determined by number of species within each category found in a given country.

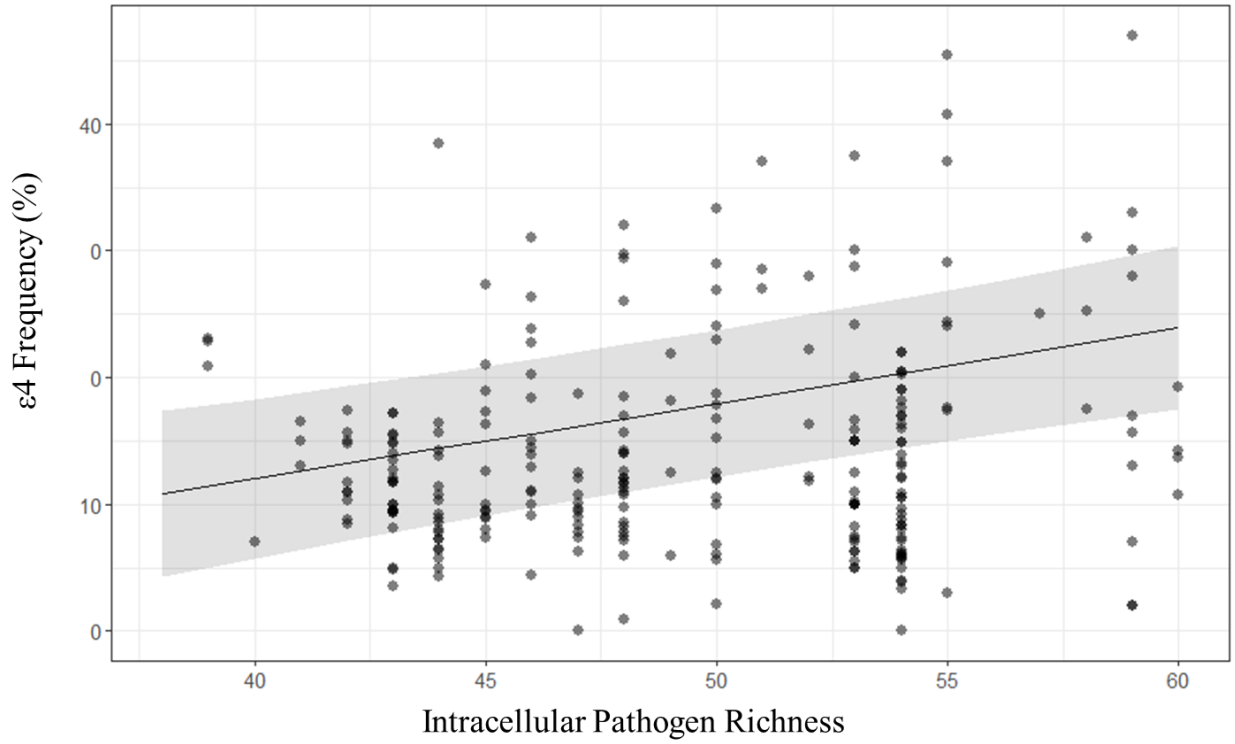

Figure 1. Relationship between APOE ε4 allele frequency and intracellular pathogen richness. Dots represent actual data points.

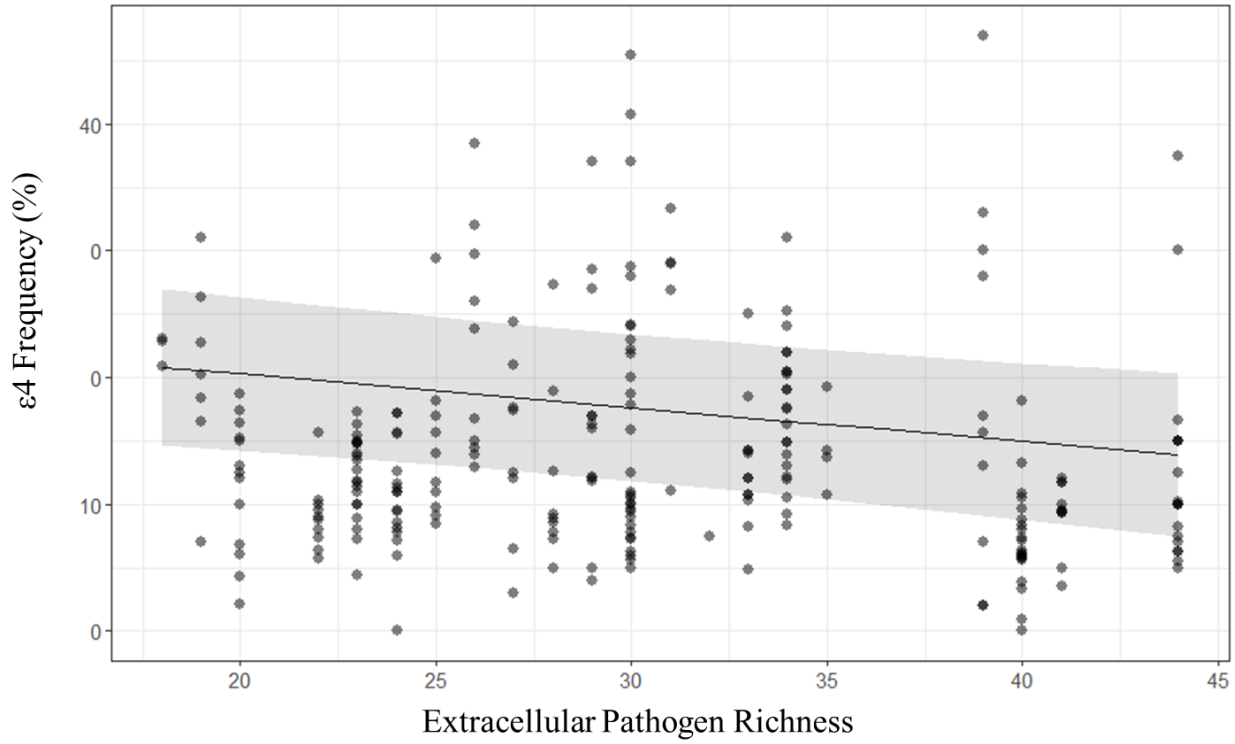

Figure 2. Relationship between APOE ε4 allele frequency and extracellular pathogen richness. Dots represent actual data points.