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APOE genotype is associated with *Ex vivo* inflammatory responses in healthy individuals: Potential implications for Alzheimer's disease

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A B S T R A C T

Background: Apolipoprotein E (*APOE*) genotype and inflammation are each linked to Alzheimer's disease (AD) risk. The *APOE-ε4* (*APOE4*) allele is associated with increased AD risk, while the *APOE-ε2* (*APOE2*) allele is protective. The mechanisms behind these associations remain unclear, particularly regarding the role of inflammation. This study examined the association between *APOE* genotype and different measures of inflammation, including mitogen-stimulated cytokine production (*ex vivo*) which provides a stable measure of immune response traits.
Methods: We examined basal and lipopolysaccharide (LPS)-stimulated cytokine levels (interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF)) and C-reactive protein (CRP) in blood samples from 91 healthy individuals (ages 18–51) of varying *APOE* genotypes.
Results: APOE4 carriers exhibited higher LPS-stimulated inflammatory responses (IL-1β, TNF-α) in peripheral whole blood compared to non-carriers, while *APOE2* carriers showed a hypo-inflammatory profile (lower stimulated IL-1β) compared to *APOE3* individuals.
Conclusion: Distinct inflammatory phenotypes were associated with *APOE* genotypes, with *APOE4* carriers exhibiting a hyper-inflammatory response and *APOE2* carriers exhibiting a hypo-inflammatory response. These profiles may contribute to differential AD risk, highlighting the potential involvement of inflammation in *APOE*-associated AD susceptibility.

1. Introduction

Apolipoprotein E (*APOE*) genotype (Corder et al., 1993; Kim et al., 2022) and inflammatory traits (S et al., 2016; Bettcher et al., 2014) are each associated with cognitive decline and Alzheimer's Disease (AD) risk. Apolipoprotein E (ApoE) is the major lipid transport protein in the central nervous system (CNS). The *APOE* gene, located on chromosome 19, has 3 allelic variations (ε2, ε3, ε4) with *APOE*-ε3 (*APOE3*) allele being the most common (Corder et al., 1993, 1994).The associations of the *APOE*-ε4 (*APOE4*) allele with increased AD risk (Corder et al., 1993; Sun et al., 2023; Yamazaki et al., 2019) and the *APOE*-ε2 (*APOE2*) allele with decreased AD risk (Kim et al., 2022; Corder et al., 1994) are well-established, however, the underlying mechanism(s) of isoform-specific CNS pathology remain unclear (Kim et al., 2022; Lin et al., 2024).

APOE4 promotes tau pathology, with increased tau phosphorylation and neurofibrillary tangle formation, partly through isoform-specific interactions of ApoE fragments with phosphorylated tau (Troutwine et al., 2022). It is also linked to increased brain Aβ burden via reduced clearance and enhanced accumulation and is associated with earlier and greater amyloid deposition (Troutwine et al., 2022; Rebeck, 2017). While amyloid-β deposition can activate microglia and trigger

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pro-inflammatory signaling, which in turn, may promote tau phosphorylation, propagation, and neurodegeneration (Parhizkar et al., 2022), neuroinflammation is increasingly recognized as a central mechanism driving neurodegeneration in AD - i.e., not merely as a secondary response to amyloid-β plaques and neurofibrillary tau tangles (Parhizkar et al., 2022). Blood–brain barrier integrity has also been shown to be reduced in *APOE4* carriers, further linking central pathology with peripheral inflammatory processes (Montagne et al., 2020).

Inflammation is often measured with inflammatory marker panels such as interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and C-reactive protein (CRP), which reflect different aspects of systemic (basal) inflammation (Majd et al., 2018; Van Bogart et al., 2021; Lindsay et al., 2022). *Ex vivo* stimulation of white blood cells with lipopolysaccharide (LPS), a bacterial endotoxin, is a well-established method for assessing the innate immune system's ability to mount an inflammatory response (Jilma-Stohlawetz et al., 2017). Whereas basal cytokine levels represent the current state of inflammatory signaling, LPS-stimulated cytokine production is highly reproducible within individuals over time and represents a relatively stable, trait-like measure of innate immune reactivity (Jorda et al., 2025; Davis et al., 2020).

Although the mechanistic details have not been fully characterized, there is evidence that ApoE plays an important role in the innate immune system (Rebeck, 2017; Keene et al., 2011; Tsoi et al., 2007; Laskowitz et al., 1997). The following animal studies suggest that while ApoE (ε2, ε3, and ε4) serves an anti-inflammatory role, *APOE4* appears to exert a less pronounced anti-inflammatory effect compared with *APOE3*. One study showed lower LPS-stimulated TNF-α levels in neuronal-glial cultures derived from ApoE-deficient mouse pups incubated with ApoE (ε3 or ε4) compared to control *APOE* deficient cultures (Laskowitz et al., 1997). Similarly, exogenous ApoE introduced to rat glial cultures induced anti-inflammatory processes (Guo et al., 2004). Moreover, E4-expressing mice glia have been shown to secrete higher levels of TNF-α compared to E2 or E3 glia when exposed to tau-expressing neurons (Shi et al., 2017). *APOE3* has also been associated with less inflammation than other *APOE* genotypes; when stimulated with LPS, *APOE3*-expressing mouse peritoneal macrophages secreted lower LPS-stimulated TNF-α and IL-6 (and expressed lower mRNA for these same proteins) compared to *APOE2* and *APOE4* macrophages (Tsoi et al., 2007). Further, *APOE4* has been associated with higher inflammation compared to *APOE3*; this is corroborated by higher levels of inflammatory cytokines in stimulated *APOE4* mouse microglia cultures (TNF-α, IL-6, IL-12p40) and macrophage cultures (TNF-α) in comparison to similarly treated *APOE3* cell controls (Vitek et al., 2009). In addition, comparing brain tissue from mice with *APOE3* vs. *APOE4* genotypes, LPS-stimulated *APOE4* mouse cortical tissue expressed higher levels of TNF-α and IL-12p40 mRNA than LPS-stimulated *APOE3* mouse cortical tissue (Vitek et al., 2009). Other studies have similarly reported higher LPS-stimulated cytokine levels in *APOE4* mice compared to *APOE3* mice (Lynch et al., 2003; Zhu et al., 2012). Although ApoE plays an anti-inflammatory role, these animal studies suggest the *APOE4* genotype is less anti-inflammatory than the *APOE3* genotype.

The role of *APOE4* in inflammation is further corroborated by a human study (n = 14) in which LPS-stimulated blood samples from *APOE4* allele positive (*APOE4*(+)) participants (n = 7) had higher levels of circulating cytokines (TNF-α, IL-6, IL-17, IL-1β, Interferon (IFN)-γ, GM-CSF and monocyte chemoattractant protein-1 [MCP1]) than *APOE4* allele negative (*APOE4*(−)) participants (Gale et al., 2014). Further, following intravenous LPS administration, *APOE4(+)* participants exhibited a greater rise in TNF-α and an earlier rise in IL-6 than *APOE4 (−)* participants (Gale et al., 2014). These findings in humans, though based on a small sample, suggest a link between APOE genotype and inflammatory phenotype.

If such an association exists, this might elucidate an immune pathway through which *APOE* genotype modifies risk for AD. To explore this possibility, this study assessed the influence of *APOE* genotype on blood levels of basal and LPS-stimulated inflammatory markers. We hypothesized that in healthy young adults, *APOE4* carriers and *APOE2* carriers would generate a different immune response to LPS stimulation compared to *APOE3* carriers.

2. Methods

2.1. Study cohort

We utilized data obtained from 91 healthy individuals from the greater New York City area (Table 1); who were initially recruited for their participation in recreational soccer as part of the Einstein Soccer Study (Lipton et al., 2013; Rubin et al., 2018) to study repetitive head impacts. Inclusion criteria were ages 18-55, amateur soccer play for at least 5 years, currently playing at least 6 months per year, and fluency in English. Exclusion criteria were schizophrenia, bipolar disorder, current substance use disorder, any drug use within 30 days, or any contraindication to magnetic resonance imaging (MRI). This study was reviewed and approved by the Albert Einstein College of Medicine IRB on 5/30/2013 (IRB # 2012-658). All participants provided written informed consent.

2.2. APOE genotypes

Participants were genotyped at enrollment as described in previous publication (Hunter et al., 2020a). Five mL of whole blood was obtained in EDTA-coated collection tubes via venipuncture. The Global Screening Array-24.v1.0 was used to genotype *APOE* gene variant rs7412 with a genotyping call rate of 98.5%; only samples with a 95% genotype call rate or more were retained for analysis. TaqMan (Thermo Fisher Scientific, Waltham, MA) was used to genotype the variant rs429358 with a

Table 1
Study Demographics. N = 91 healthy participants recruited from the NYC area.

		Cohort Stratified by *APOE4* allele		
		E4 –	E4 +	p-value
Number of participants (% of cohort)		68 (74.7%)	23 (25.3%)	–
Age (years) Mean (Std.)		25.13 (7.31)	25.09 (6.25)	0.354
APOE genotype (% of cohort)	E2/E3	10 (10.9%)	–	–
	E3/E3	58 (63.7%)	–	–
	E4/E3	–	22 (24.1%)	–
	E4/E4	–	1 (1.1%)	–
Sex (% of cohort)	Female	18 (19.8%)	8 (8.8%)	0.439
Race (% of cohort)	White	44 (48.4%)	12 (13.2%)	0.339
	Black	12 (13.2%)	6 (6.6%)	
	Asian	4 (4.4%)	0 (0.0%)	
	Declined to Answer	8 (8.8%)	5 (5.5%)	
Ethnicity (% of cohort)	Non-Hispanic	50 (54.9%)	14 (15.4%)	0.275
	Hispanic	16 (17.6%)	9 (9.9%)	
	Declined to Answer	2 (2.2%)	0 (0.0%)	
Race/Ethnicity (% of cohort)	White/Non-Hispanic	37 (40.7%)	9 (9.9%)	0.235
	Other	31 (34.1%)	14 (15.4%)	–
BMI (kg/m^2) Mean (Std.)		24.34 (3.38)	25.26 (3.67)	0.251

call rate of 99.3%. The TaqMan assay was used for rs429358 because a higher genotyping error rate than the Global Screening Array chip was found in our quality control step using samples with previously known *APOE* genotypes. Genotype data were analyzed with the Golden Helix SVS software (Golden Helix, Bozeman, MT). The genotypes did not deviate from the Hardy-Weinberg equilibrium.

2.3. Inflammation

Basal and LPS-stimulated cytokines (IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, GM-CSF) and high sensitivity CRP were assessed from blood and quantified using V-Plex protein arrays purchased from Meso Diagnostics (Rockville, MD) and following the manufacturer's protocol. LPS stimulation was performed on a 1 mL subsample of whole blood shortly after collection. The blood was incubated with LPS (1 μg/mL, *E. coli* 055:B5, Sigma Aldrich, Newark, DE) on a rotational shaker at 37°C with 5% CO_2 for 2 h. Following incubation, the samples were centrifuged at 1500 g for 15 min, and the supernatant was aliquoted and stored at −80°C until analysis. The minimum detection limit for all cytokines (basal and stimulated) ranged between 0.03 and 0.14 pg/mL and for CRP was 46.9 pg/ml. All samples were run in duplicate and samples with coefficients of variation (CV) > 15% were rerun.

2.4. Statistical analysis

Participants (n = 91) were stratified by APOE4 allele status. Following precedent (Van Bogart et al., 2021; Graham-Engeland, 2018) and to account for skewness of inflammation data, logarithmic (log) transformation was applied, using a log formula of (x+1), after which data >3 standard deviations above the mean were winsorized to 3 standard deviations (SD) from the mean. The distribution of inflammation data (log (μg/mL)) was plotted and assessed for normality using the Shapiro-Wilkes test (Supplemental Fig. 1 and Supplemental Table 1). The number of winsorized values are reported in Supplemental Table 2.

Following precedent (Van Bogart et al., 2021), biological sex was included as a binary covariate and race/ethnicity was included as a binary covariate categorized as Non-Hispanic White (n = 46) or "Other" (n = 45). The "Other" category was a non-homogenous group, in which all participants who did not identify as "White" and "Non-Hispanic" were grouped (Table 1). Race/ethnicity was included as a covariate because prior studies, have demonstrated racial differences in biomarkers associated with Alzheimer's disease and related dementias (García et al., 2026). We compared demographic characteristics between *APOE4*(+) and *APOE4*(−) groups using Kruskal-Wallis tests for continuous variables and Fisher's Exact tests for categorical variables.

All statistical tests were performed in R 4.2.1 (Team, 2022). APOE4 (+) denotes carriers of at least one ε4 allele (e.g., ε3/ε4, ε4/ε4), while APOE4(−) denotes non-carriers. We first performed principal component (PC) analysis on the inflammation panels to gain insight about potential correlations within the dataset; we then tested for associations of *APOE4* and PCs that explained relatively high variance (2.4.1). Next, guided by the results of the PC analysis (PCA), we individually probed the loadings of PCs associated with *APOE4* using linear regression models (2.4.2.a). We then further stratified the cohort into *E2/E3* (n = 10), *E3/E3* (n = 58) and *E4/–* (n = 23) individuals (2.4.2.b) to compare *E2/E3* with *E3/E3* and *E4/–* with *E3/E3*.

2.4.1. Principal component analysis (PCA)

PCA was performed using the R package FactoMineR (Lê et al., 2008) on the set of basal biomarkers (IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, GM-CSF, CRP) and the set of LPS-stimulated cytokines (IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, GM-CSF). The association of the *APOE4* allele and the first two PCs (PC1 or PC2) was tested using a linear model; biological sex, chronological age, race/ethnicity, and BMI were included as covariates.

2.4.2. Linear regression models

APOE4(+) and *APOE4*(−) stratified analysis: Let A_i denote the *APOE4* presence in each individual *i*. I_i denotes the log-scale cytokine level (log(μg/mL)) of a given cytokine (IL-1β, IL-4, IL-6, IL-8, IL-10, or TNF-α) for one participant *i*. The association of *APOE4* allele with LPS-stimulated cytokine PC1 loadings (IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α) was tested using a linear model (Equation (1)); biological sex, chronological age, race/ethnicity, and BMI are included as covariates (C_i). β_0 denotes intercept, and coefficient β_{APOE4} denotes the independent cross-sectional association of *APOE4* with the cytokine level. e_i is a random error term.

$$I_i = \beta_0 + \beta_{APOE4} A_i + \zeta C_i + e_i \quad (1)$$

E2/E3, *E3/E3*, and *E4/–*stratified analysis: Now let A_i denote the *APOE* genotype (*E2/E3*, *E3/E3*, or *E4/–*) in each individual *i*. I_i denotes the log-scale cytokine level (log(μg/mL)) of a given cytokine (IL-1β, IL-4, IL-6, IL-8, IL-10, or TNF-α) for one participant *i*. The association of *APOE4* genotype with LPS-stimulated cytokine PC1 loadings (IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α) was tested using a linear model (Equation (2)); biological sex, chronological age, race/ethnicity, and BMI are included as covariates (C_i). β_0 denotes intercept, and coefficient $\beta_{APOE-genotype}$ denotes the independent cross-sectional association of APOE genotype with the cytokine level. e_i is a random error term.

$$I_i = \beta_0 + \beta_{APOE-genotype} A_i + \zeta C_i + e_i \quad (2)$$

3. Results

Our study included 91 healthy participants (ages 18-51; 29% female; 50.5% Non-Hispanic White) with 23 *APOE4* (+) participants (n = 22 *E3/E4; n=1 E4/E4*) and 68 *APOE4*(−) participants (n = 58 *E3/E3*; n = 10 *E2/E3*) (Table 1).

3.1. PCA showed association of APOE4 with elevated LPS-stimulated cytokines

Separate PCAs were performed on the panels of basal cytokines (Fig. 1) and LPS-stimulated cytokines (Fig. 2). The *APOE4* allele was not associated with basal cytokines for either PC1 or PC2 (Fig. 1). Conversely, the heatmap of LPS-stimulated cytokine PCA loadings (Fig. 2a) indicates that LPS-stimulated cytokines (IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α) had strong positive loadings in PC1, with red representing positive loadings and blue representing negative loadings. The scree plot (Fig. 2b) shows that PC1 accounted for over 60% of the variance in the cytokine data. Individuals with the *APOE4* allele (n = 23) exhibited higher PC1 scores compared to those with E3/E3 or E2/E3 genotypes (n = 68) (Fig. 2c). In contrast, PC2 did not show an association with the *APOE4* allele (Fig. 2d).

3.2. APOE4 allele was associated with higher LPS-stimulated IL-1β and TNF-α

In comparison to *E3/E3* and *E2/E3* individuals (see methods 2.4.2.a), the *E4/–* genotype was associated with higher levels of LPS-stimulated IL-1β ($\beta_{E4\text{-allele}} = 0.587$; $p < 0.05$) and TNF-α ($\beta_{E4\text{-allele}} = 0.351$; $p < 0.05$) (Table 2, Fig. 3). Additionally, higher LPS-stimulated IL-1β ($\beta_{Race\text{-}Eth} = 0.517$; $p < 0.05$) was associated with identifying as Non-Hispanic White, which is one of the covariates included in our analysis (Table 2).

3.3. APOE2 allele was associated with lower LPS-stimulated IL-1β compared to APOE3

In comparison to *E3/E3* individuals (see methods 2.4.2.b), the *E2/E3* genotype was associated with lower stimulated IL-1β levels ($\beta_{E2\text{-allele}} = -0.817$; $p < 0.05$) (Table 3, Fig. 4). In this same analysis (see

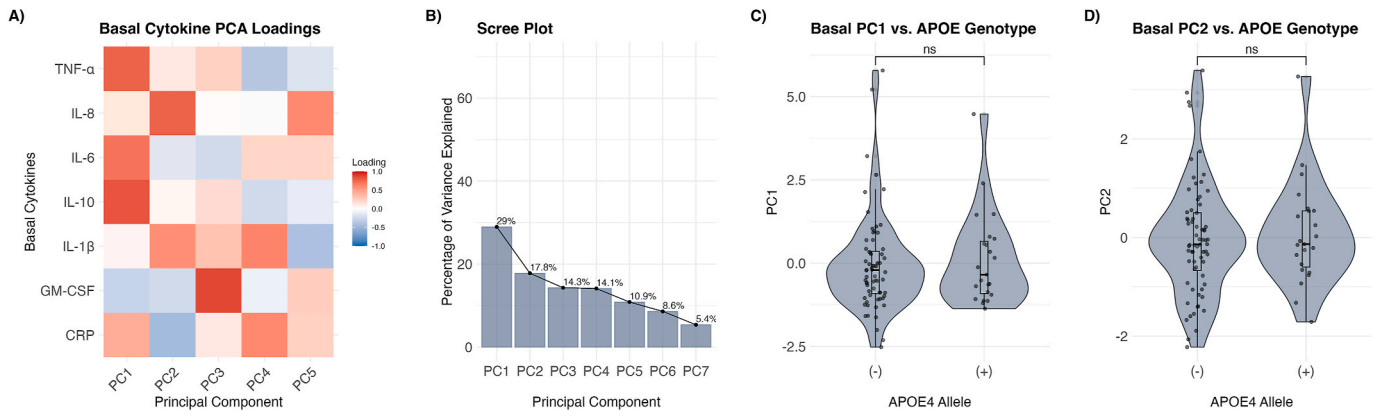

Fig. 1. Principle Component Analysis of Heparin-stimulated cytokines. A) Heatmap of PCA loadings, red signifying positive loading and blue signifying negative loading. **B)** Scree Plot shows variance explained by each PC. **C)** PC1 stratified by *APOE* genotype. (+) denotes the genotype *E4/–* (n = 23). (−) denotes an *E3/E3* or *E2/E2* genotype (n = 68). PC1 was not associated with the *E4* allele. **D)** PC2 stratified by *APOE* genotype. PC2 was not associated with the *E4* allele. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

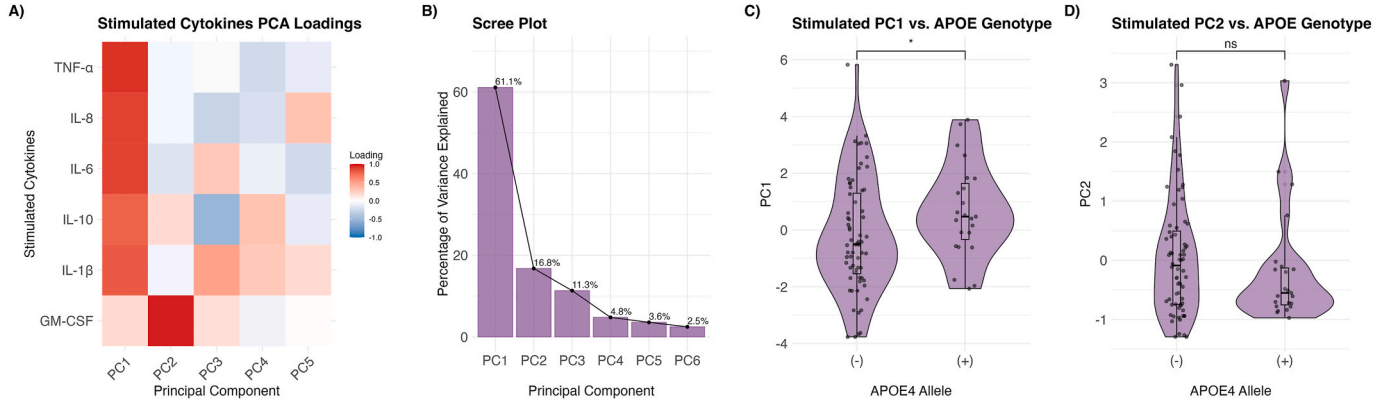

Fig. 2. Principle Component Analysis of LPS-stimulated cytokines. A) Heatmap of PCA loadings, red signifying positive loading and blue signifying negative loading. Strong positive loadings in PC1 for all LPS-stimulated cytokines. **B)** Scree Plot shows variance explained by each PC. >60% of variance can be explained by PC1. **C)** PC1 stratified by *APOE* genotype. (+) denotes the genotype *E4/–* (n = 23). (−) denotes an *E3/E3* or *E2/E2* genotype (n = 68). The *E4* allele was associated with higher PC1 which had positive cytokine loadings. **D)** PC2 stratified by *APOE* genotype. PC2 was not associated with the *E4* allele. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Linear regression model estimates the association of *E4* allele with LPS-stimulated cytokine levels (IL-10, IL-6, IL-1β,IL-8, TNF-α). Covariates include biological sex, chronological age, race-ethnicity as a binary factor, and BMI. P-values <0.05 are bolded. The *E4* allele is associated with higher IL-1β and TNF-α levels. Identifying as White/non-Hispanic is associated with higher IL-1β levels.

LPS-Stimulated Cytokines	$\beta_{E4\text{-allele}}$	p-value	$\beta_{Biological\ Sex}$	p-value	β_{Age}	p-value	$\beta_{Race\text{-}Ethnicity}$	p-value	β_{BMI}	p-value
IL-10	0.309	0.160	−0.0184	0.932	−0.0124	0.363	0.212	0.292	0.0239	0.383
IL-6	0.539	0.0503	0.120	0.656	−0.0256	0.134	0.190	0.446	−0.0111	0.743
IL-1β	0.587	**0.019**	0.378	0.123	−0.0296	0.0565	0.517	**0.0238**	−0.0105	0.733
IL-8	0.227	0.260	0.0420	0.833	−0.0153	0.223	0.0394	0.831	0.0406	0.108
TNF-α	0.351	**0.0427**	0.156	0.358	−0.0139	0.193	0.162	0.301	0.0181	0.396

methods 2.4.2.b), in comparison to only *E3/E3* individuals, the *E4/–* genotype was not associated with higher LPS-stimulated cytokines.

3.4. Supplemental analyses

We studied healthy adult soccer players, who may be more fit than non-athletes and some of whom have a history of sport-related repetitive head impacts (RHI) and/or concussion. In our supplemental analysis, which included RHI and concussion history as covariates, we found that prior concussion history was associated with lower IL-1β ($\beta_{concuss} = -0.490$; $p < 0.05$) and TNF-α ($\beta_{concuss} = -0.375$; $p < 0.05$) (Supplemental Table 3). We did not find any association of RHI with cytokine levels (Supplemental Table 3). Notably, these findings recapitulated that the *APOE4* allele was associated with higher IL-1β and TNF-α. Covarying for RHI and concussion, the *APOE4* allele was also found to be associated with higher IL-6 ($\beta_{E4\text{-allele}} = 0.572$; $p < 0.05$).

In a post-hoc analysis, we confirmed that without winsorization, basal cytokine PC1 was not associated with APOE ($p = 0.42$). Thus, our finding that basal cytokine levels are not associated with APOE genotype is not an artifact of winsorization. None of the LPS-stimulated cytokine values exceeded the 3 SD winsorization threshold. Therefore, our findings with stimulated cytokines are not impacted by winsorization.

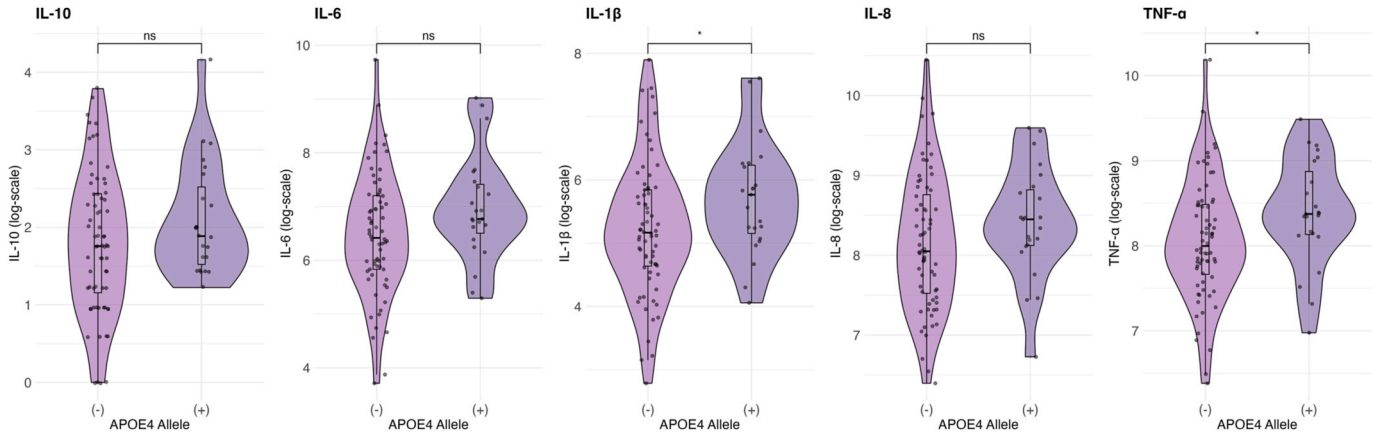

Fig. 3. LPS-stimulated cytokine levels stratified by presence of *E4* allele. Violin plots describe the distribution of cytokine levels (from left to right: IL-10, IL-6, IL-1β, IL-8, TNF-α), stratified by the presence (+) of *E4* allele. (*) denotes a significant group effect ($p < 0.05$) after accounting for covariates (i.e., biological sex, chronological age, BMI, and race-ethnicity). The *E4* allele was associated with IL-1β, and TNF-α levels.

Table 3
Linear regression model estimates the association of *APOE* genotype with LPS-stimulated cytokine levels (IL-1β, IL-6, IL-8, IL-10, TNF-α). Participants are stratified as *E2/E3*, E3/E3 and E4/–. Covariates include biological sex, chronological age, race-ethnicity as a binary factor, and BMI. P-values <0.05 are bolded. The *E2* allele was associated with lower IL-1β levels. Identifying as White/non-Hispanic was associated with higher IL-1β levels.

LPS-Stimulated Cytokines	$\beta_{E2\text{-allele}}$	p-value	$\beta_{E4\text{-allele}}$	p-value	$\beta_{Biological\ Sex}$	p-value	β_{Age}	p-value	$\beta_{Race\text{-}Ethnicity}$	p-value	β_{BMI}	p-value
IL-10	−0.153	0.618	0.289	0.200	−0.0134	0.951	−0.0130	0.346	0.212	0.293	0.022	0.416
IL-6	−0.416	0.276	0.482	0.0841	0.133	0.620	−0.0271	0.114	0.191	0.443	−0.0150	0.661
IL-1β	−0.817	**0.0166**	0.476	0.0533	0.404	0.0906	−0.0325	0.0321	0.518	**0.0198**	−0.0180	0.549
IL-8	0.0380	0.894	0.233	0.261	0.0408	0.839	−0.0152	0.232	0.0393	0.832	0.0410	0.109
TNF-α	−0.0937	0.697	0.338	0.0559	0.159	0.352	−0.0143	0.186	0.162	0.303	0.0173	0.423

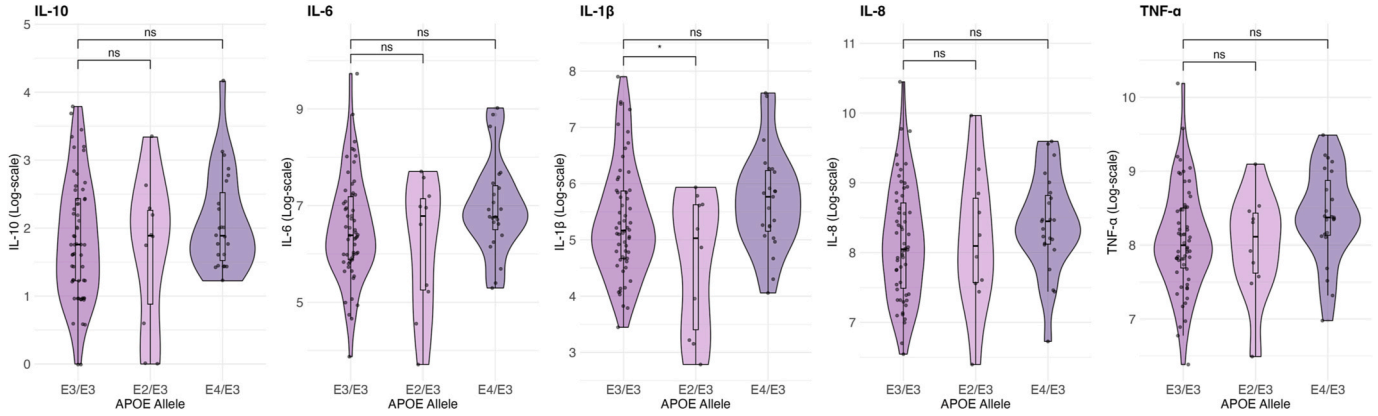

Fig. 4. LPS-stimulated cytokine levels stratified by *APOE* genotype. Violin plots describe the distribution of cytokine levels (from left to right: IL-10, IL-6, IL-1β, IL-8, TNF-α), stratified by *APOE* genotype (*E2/E3*, *E3/E3,* and *E4/–*). (*) denotes a significant group effect ($p < 0.05$) after accounting for covariates (i.e., biological sex, chronological age, BMI, and race-ethnicity). The *E2* allele was associated with lower IL-1β levels.

4. Discussion

4.1. APOE genotypes are associated with differential innate inflammatory response profiles

We directly examined associations of *APOE* genotype with inflammatory profiles in healthy young individuals and found associations of *APOE* genotype with cytokine levels that were specific to LPS-stimulated responses. LPS-stimulated cytokines represent trait responsiveness of the innate immune system and index an individual's capacity to generate an immune response to mitogenic challenge. The lack of association of APOE genotype with basal cytokine levels, alongside its observed associations with stimulated cytokine levels, suggests that APOE genotype is more strongly related to trait inflammatory responsiveness than to basal inflammatory status. Notably, in LPS-stimulated blood, *APOE4*(+) individuals exhibited a stronger inflammatory response (i.e., greater TNF-α and IL-1β) compared to non-carriers, while *APOE2*(+) individuals displayed a weaker inflammatory response (i.e., lower IL-1β) compared to the *E3/E3* genotype. These results support existing literature suggesting that *APOE4* is linked to heightened inflammatory activity (Guo et al., 2004; Vitek et al., 2009; Lynch et al., 2003; Zhu et al., 2012; Gale et al., 2014). Our results identify distinct inflammatory phenotypes associated with *APOE* alleles, with the *APOE2* allele exhibiting a hypo-inflammatory profile compared to the *E3/E3* genotype and the *APOE4* allele displaying a hyper-inflammatory profile relative to APOE4 noncarriers.

A hyper-inflammatory peripheral immune phenotype in the *APOE4* cohort suggests a greater likelihood of heightened neuroinflammatory

responses, potentially priming microglia toward a pro-inflammatory M1 state (Wang et al., 2023). Such a shift has been shown to reduce amyloid-β clearance compared to the reparative M2 state (Wang et al., 2023), and to contribute to amyloid accumulation and neuronal damage (d'Errico et al., 2022; Fruhwürth et al., 2024; Leng et al., 2021). Similarly, a hypo-inflammatory peripheral immune phenotype in the *APOE2* cohort may indicate a less inflammatory central state. While speculative, we postulate such a mechanism could partially explain both the known increased AD risk observed with the *APOE4* genotype and decreased AD risk with the *APOE2* genotype. Furthermore, it is known that individuals with the *APOE4* genotype have an increased risk for amyloid-related imaging abnormalities-edema (ARIA-E), where cerebral edema develops following anti-amyloid protofibril immunotherapy (e.g., Lecanemab), putatively representing a hyper-inflammatory response (Cummings et al., 2023). ARIA-E could thus be related to an association of *APOE4* with a heightened central inflammatory response (i.e., neuroinflammation).

Beyond AD, APOE genotype has been implicated in a range of inflammatory conditions, including repetitive head impacts (Hunter et al., 2020b) and chronic traumatic encephalopathy (Atherton et al., 2022), sepsis (Atherton et al., 2022), and COVID-19 (Safdari Lord, 2022)-where dysregulated immune responses and central inflammation contribute to disease severity. The genotype-associated differences in peripheral inflammatory responses observed in our study may therefore reflect broader immune response phenotypes that influence susceptibility and outcomes across diverse disease states, including those affecting younger populations.

Future empirical confirmation of the hypotheses raised by our findings are needed to determine if neuroinflammatory response magnitudes, and perhaps microglial functional states, vary by *APOE* genotype.

4.2. Limitations and future research

The interpretation of these results should consider several limitations including sample size, in particular the small number of *APOE2*-positive participants (n = 10). Only two studies have reported on *APOE2* inflammatory profiles (Tsoi et al., 2007; Zhu et al., 2012). One study reported *higher* TNF-α and IL-6 in stimulated *APOE2* mouse peritoneal macrophages compared to *APOE3* (Tsoi et al., 2007). The other study treated the APOE2 group as a control and reported prolonged increases of LPS-stimulated IL-1β, IL-6 and TNF-α in *APOE4* mice compared to both *APOE2* and *APOE3* mice (Zhu et al., 2012). These studies further highlight that more research is needed, particularly in human participants. To fully describe the inflammatory profiles of the *APOE*-isoforms, future studies should include larger numbers of *APOE2*(+) participants.

This study was conducted in a relatively young, healthy cohort without the assessment of AD biomarkers (e.g., beta-amyloid). Therefore, the findings should not be interpreted to reflect AD-related pathology. Rather, the observed associations are best understood as reflecting APOE genotype-related differences in inflammatory responsiveness; this, in turn, may have broader relevance across the lifespan and in diverse disease contexts. Future studies in older populations with neurodegenerative biomarker characterization (e.g., beta-amyloid, tau) will be important to further define the relevance of our findings to other indices of neuroinflammation and AD.

Several factors not assessed in this study may influence inflammatory responses and interact with APOE genotype, and should be examined in future studies. These include lifestyle variables (e.g., diet, physical activity, and medication use), cardiometabolic risk, along with behavioral and cognitive measures. Future studies that enroll more diverse samples would increase the generalizability of these findings.

A previous study (n = 11,675) probed *APOE* associations with CRP and found lower CRP to be associated with *APOE4* (Wang et al., 2022). We did not find an association of APOE with basal inflammation (Fig. 1) and on supplemental analysis found no significant association of the *APOE4* allele with CRP (Estimate = −0.0105; p = 0.71175). The directionality of the effect we found corroborates prior literature (Wang et al., 2022) and the lack of statistical significance might be attributed to a much smaller sample size.

5. Conclusion

Prior research has described an association between *APOE4* and a hyper-inflammatory phenotype in cell culture (Tsoi et al., 2007; Laskowitz et al., 1997; Vitek et al., 2009), animal models (Vitek et al., 2009) and in a small sample of human subjects (n = 14) (Gale et al., 2014). Here we demonstrate this phenomenon in 91 young healthy human participants. Our results further suggest that a hypo-inflammatory phenotype occurs in *APOE2*(+) individuals, which has not yet been reported and requires follow-up with a larger sample size. These results support a phenotypic mechanism involving *APOE* genotype-associated inflammation, which may help to explain the known link of *APOE* genotypes with AD risk. Characterizing *APOE*--differentiated immune responses in humans is important to elucidate key molecular pathways connecting *APOE* with AD, and to better understand the risk factors and associated pathways connected to AD pathology.

Ethics declarations

This study was reviewed and approved by the Albert Einstein College of Medicine IRB on 5/30/2013 (IRB # 2012-658). All participants provided written informed consent.

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CRediT authorship contribution statement

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Declaration of competing interest

There is no conflict of interest among all authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2026.101307.

Data availability

Data will be made available on request.

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