

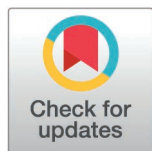
RESEARCH ARTICLE

Divergent biological consequences of APOE isoforms across industrialized and non-industrial environments

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Data availability statement: The OA HeLP and THGP prioritize minimizing risk to study participants and follow the CARE Principles for Indigenous Data Governance (emphasizing Collective Benefit, Authority to Control, Responsibility, and Ethics) and the FAIR

Abstract

The apolipoprotein $\epsilon 4$ (APOE $\epsilon 4$) isoform directly alters cholesterol and immune biology and is associated with an increased risk of neurodegenerative and cardiometabolic disease in industrialized settings; nevertheless, APOE $\epsilon 4$ —which is ancestral in humans—has persisted over evolutionary time. One potential explanation is that the costs and benefits of APOE $\epsilon 4$ were significantly different in the environments in which humans evolved compared to those we experience today. In support, previous work has suggested that living in a high pathogen environment, engaging in high levels of physical activity, or eating a low fat diet can dampen the detrimental effects of APOE $\epsilon 4$, and has revealed positive effects for fertility. However, direct tests of whether APOE isoforms are associated with different biological outcomes in non-industrial versus industrialized contexts are lacking. Working with the Turkana of Kenya and the Orang Asli of Peninsular Malaysia—two Indigenous groups in which individuals of shared ancestry span a continuum of subsistence, non-industrial to urban, industrialized lifestyles—we investigated how APOE genotypes impact cholesterol, immunological, and reproductive traits and directly tested for genotype x environment (GxE) interactions. First, we confirmed

Guiding Principles for scientific data (emphasizing Findability, Accessibility, Interoperability, and Reusability). Individual-level data are stored in a public but protected repository at Zenodo (10.5281/zenodo.18686419) and available through restricted access. Requests for de-identified data must include a detailed application and procedures for data security, privacy, and minimizing potential harm. Sample data use agreements are provided at <https://lea-lab.org/resources.html>. Code to perform all analyses described here is available on GitHub as follows: 1) generating the urbanicity score for both populations (https://github.com/mwatowich/Multi-population_lifestyle_scales); 2) performing statistical analyses and processing the accelerometry data (https://github.com/mwatowich/effects_of_APOE_across_industrialization_gradient); 3) processing the Orang Asli transcriptomic data (<https://github.com/laylabrassington/ELvsCL>).

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established genotype effects across lifestyles, showing that having more APOE $\epsilon 4$ alleles is associated with higher total cholesterol, higher LDL cholesterol, and lower HDL cholesterol. Second, we tested for lifestyle interactions, finding lifestyle-dependent effects of genotype on innate immune biomarkers in the Orang Asli but not Turkana. Finally, we show that having more APOE $\epsilon 4$ alleles is correlated with an extended reproductive lifespan, however this effect is relatively weak, is not consistent across populations, and does not correspond with a higher reproductive output. Together, our study provides evidence that industrialized environments can modify the biology of APOE $\epsilon 4$; however, we find that APOE $\epsilon 4$ is not universally beneficial in non-industrial contexts, highlighting the role of local environmental variation in determining its specific costs and benefits.

Author summary

The apolipoprotein $\epsilon 4$ allele is a major risk factor for Alzheimer's dementia risk in industrialized countries, yet it persists at moderate frequencies around the world without strong evidence of being selected against. One explanation for this paradox is that $\epsilon 4$ is beneficial in certain environments—especially those more similar to the non-industrial contexts in which humans evolved—an idea which is encouraged by observations that APOE influences biological processes from cholesterol processing to immune function. Indeed, prior studies in non-industrial settings have found immune and fertility benefits of $\epsilon 4$. We worked with two Indigenous groups who span wide lifestyle gradients—the Turkana of Kenya and the Orang Asli of Peninsular Malaysia—to study how APOE genotypes affect cholesterol, immunological, and reproductive traits across industrialization. First, as expected, we found that $\epsilon 4$ was strongly associated with higher LDL and total cholesterol levels. Second, we found weak but consistent evidence that $\epsilon 4$ was associated with lower systemic inflammation and mixed evidence that this effect was stronger in industrialized settings. Finally, we found little evidence that $\epsilon 4$ influenced female reproductive traits. Our results suggest that the effects of APOE $\epsilon 4$, and the other APOE genotypes, are consistent on cholesterol but highly context dependent on other traits.

Introduction

Humans maintain 3 isoforms of the Apolipoprotein E protein—APOE $\epsilon 4$, APOE $\epsilon 3$, and APOE $\epsilon 2$ —which each confer distinct and pleiotropic effects on cholesterol and innate immune traits [1,2]. APOE $\epsilon 4$ is well known as a major disease risk marker: in urban, industrialized settings, individuals homozygous for the $\epsilon 4$ isoform have a ~12-fold higher risk of Alzheimer's disease, a ~1.5-fold higher risk of cardiovascular diseases (CVD), and an overall higher risk of premature death [3–6]. However, the APOE $\epsilon 4$ allele is considered ancestral in humans and shows minimal evidence of being selected against, raising the question of why it persists despite clear costs [7,8].

Two evolutionary theories of aging may explain the persistence of APOE ϵ 4: first, that APOE ϵ 4's harmful effects are sufficiently concentrated at older ages in the "selection shadow" (consistent with mutation accumulation theory), and second, that APOE ϵ 4 may provide benefits during reproductive years that outweigh its later life costs (consistent with antagonistic pleiotropy theory) [9–11]. A third, non-mutually exclusive hypothesis is that the costs and benefits of APOE ϵ 4 are significantly different in modern, industrialized environments relative to those in which humans evolved, hindering our appraisal of its adaptive value [12–15]. In support of this third hypothesis, recent work has suggested that APOE ϵ 4 can be less detrimental under preindustrial conditions: for example, in the presence of high pathogen loads, high physical activity levels, or a non-processed, anti-inflammatory diet, APOE ϵ 4 has weaker effects on risk of both CVD and cognitive impairment [16–22]. Additionally, work with Tsimane forager-horticulturalists in Bolivia suggests that APOE ϵ 4 may even provide benefits to fertility and cognition in non-industrial, energy-limited, high pathogen settings, contrary to what has been observed in industrialized environments [17,23–26]. Although recent work across populations (e.g., studies of the Tsimane versus the US) points toward greater benefits and dampened costs of APOE ϵ 4 in non-industrial relative to industrialized environments, more direct tests for such genotype $\times$ environment interactions within populations are generally lacking.

The APOE protein is predominately produced in the liver, but the gene is also expressed by immune (e.g., macrophages) and neuronal (e.g., microglia, astrocytes) cell types [27]. The primary role of APOE is as a lipid transporter, binding to cholesterol and other phospholipids and delivering them throughout the body. Each APOE isoform has distinct cholesterol affinities: APOE ϵ 2 and to a lesser extent APOE ϵ 3 preferentially bind to cholesterol-rich, high-density lipoproteins (HDL), whereas APOE ϵ 4 preferentially binds to triglyceride-rich, very low-density lipoproteins (VLDL) [28]. APOE ϵ 4 also exhibits faster turnover of VLDL to LDL, but less effective clearance of cholesterol from circulation, resulting in higher amounts of total cholesterol and LDL in circulation for APOE ϵ 4 as compared to APOE ϵ 3 and especially APOE ϵ 2 carriers [14,29,30]. Each APOE isoform also has distinct effects on immune function: APOE ϵ 4 has been associated with baseline downregulation of the innate immune system in industrialized settings (e.g., lower CRP, IL6, and TNF- α levels) [31–34]) but stronger activation when engaged [35]. Further, relative to APOE ϵ 2 and APOE ϵ 3, the APOE ϵ 4 isoform confers decreased risk of giardia, cryptosporidium, and hepatitis B and C, but increased susceptibility to HSV-1, *C. pneumoniae*, and HIV [36–38]. However, growing evidence suggests that many of these effects—especially on innate immune activity—are highly context dependent. For example, ϵ 4 promotes macrophage-driven inflammation and atherosclerosis progression, but these effects appear to be exacerbated by excessive fatty acid circulation; similarly, Alzheimer's disease progression in ϵ 4 carriers is dependent on chronic inflammation, sex, and neutrophil-microglia interactions [33,39–42].

Several ideas have been proposed to explain why APOE ϵ 4-associated cholesterol and/or immune traits may have been beneficial throughout human evolution. First, increased cholesterol availability could have been helpful in metabolically demanding thermal environments, such as high altitudes or latitudes, though evidence for this idea is mixed [43]. Second and more supported, APOE ϵ 4 may provide several important immune benefits that are obscured or perturbed in industrialized environments: 1) cholesterol is both a component of immune cell membranes and a modulator of inflammatory signaling, such that increased circulating cholesterol could fuel immune responses in energy-limited, non-industrial environments [16,26,44,45]; and 2) the rapid and intense immune engagement characteristic of APOE ϵ 4 carriers may be beneficial in high pathogen settings, but detrimental in industrialized environments if chronic stimulation is also induced by obesity, diet, or other sterile sources of damage-associated molecular patterns [16,46–50]. Finally, some combination of APOE ϵ 4 carrier's cholesterol and immune traits may create a fertility advantage in non-industrial contexts. Though the specific mechanism is unknown, APOE ϵ 4 has been associated with earlier reproduction, shorter interbirth intervals, and greater lifetime reproductive success in energy-limited, high-pathogen settings in Bolivia, Ghana, and Ecuador [23–25]. Importantly, these same fertility benefits have not been observed in similar-sized studies of industrialized cohorts (reviewed in [23]). Taken together, these findings highlight the need to understand how APOE isoforms impact cholesterol, immune function, and fertility in non-industrial environments relevant to human evolution, and further, to directly test if these relationships are altered by industrialized conditions [51].

By working with two non-industrial populations that are experiencing rapid but heterogeneous lifestyle change, our goal is to test how exposure to industrialized, urban lifestyles alters the biological consequences of APOE isoforms within a common genetic background [52,53]. Specifically, we worked with Turkana pastoralists of Northern Kenya and Orang Asli hunter-gatherers and horticulturalists of Peninsular Malaysia, two Indigenous groups living across a recently formed gradient of non-industrial to industrialized environments [54–57]. As part of long-term anthropological and biomedical work with each group, we collected data on APOE genotypes as well as cholesterol (total, LDL, and HDL levels), innate immune (5-part white blood cell differentials, CRP and cytokine levels, blood transcriptomes), and fertility traits (age at menarche, age at first reproduction, age-specific fertility, completed fertility, age at menopause) for 1966 individuals (1105 Turkana and 861 Orang Asli). These data were collected across a comparable, continuous, well-characterized gradient from remote, small-scale, subsistence-based communities to highly urban, industrialized, and market-integrated locations [55]. Using parallel datasets from both groups, we tested the predictions that: 1) regardless of lifestyle, APOE $\epsilon 4$ would predict higher total and LDL cholesterol levels, but that 2) industrialized lifestyles might alter APOE genotype-innate immune trait associations. Finally, we tested for 3) lifestyle-dependent effects of APOE genotype on female fertility outcomes, with the prediction that APOE $\epsilon 4$ carriers would experience greater benefits in non-industrial environments (Fig 1). Together, these analyses provide a rare opportunity to test for GxE interactions within two independent groups spanning an industrialized lifestyle gradient, allowing us to evaluate the evolutionary significance of a major disease-associated genetic locus.

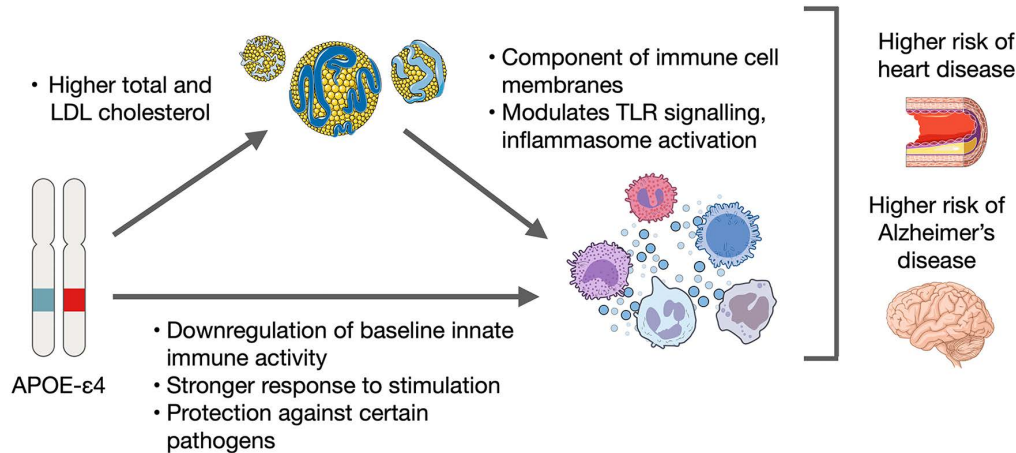
Results

Lifestyle variation provides opportunities to test for genotype x environment interactions

We drew on questionnaire, immune, cholesterol, and fertility data from two long-term projects: the Turkana Health and Genomics Project (THGP) and the Orang Asli Health and Lifeways Project (OA HeLP) [54,59] (Fig 2A and Fig A in S1 Appendix). Both projects work with communities that span a gradient from non-industrial, remote-living, subsistence-based to urban, market-integrated, industrialized environments. Briefly, the Turkana are traditionally nomadic pastoralists living in arid regions of Northwest Kenya, but increasing droughts, expansion of markets, and changing economy in Kenya has led to an increased reliance on wage labor and urban infrastructure in recent decades. The term “Orang Asli” refers to 19 ethnolinguistic groups from three major subgroups (Senoi, Negrito, Proto-Malay) that together comprise the Indigenous peoples of Peninsular Malaysia. Orang Asli have traditionally practiced a variety of subsistence strategies including hunting, gathering, horticulture, and fishing, but due especially to ongoing deforestation and government-led resettlement initiatives, many Orang Asli are now highly market-integrated and more focused on wage labor.

To operationalize this lifestyle variation, our group has previously adapted an existing measure of “urbanicity” [60] that sums information about population density, average access to infrastructure (electricity, sewage), ownership of market-derived goods (televisions, mobile phones), subsistence activities, and education access within a given location [55] (Fig 2B, urbanicity scale construction is detailed in Supplementary Methods). While we refer to this measure as an “urbanicity” index (because this is the term used in the original publication [60]) we emphasize that it is meant to capture how industrialized and market-integrated each individual’s lifestyle is—which in our sample includes fully non-industrial lifestyles—and is not equivalent to rural-urban gradients in high income countries. In total, this study included 1105 Turkana participants from 67 locations (57% female, 43% male) and 861 Orang Asli participants from 30 locations (62% female, 38% male; Fig 2C and Tables A-B in S2 Appendix). These locations spanned a similar, wide range of urbanicity values in Kenya and Malaysia (Fig 2D). Importantly, we have previously shown that urbanicity correlates with relevant aspects of energy balance (Fig 1): individuals living in locations of higher urbanicity have significantly lower physical activity levels in Orang Asli as well as greater access to processed foods, higher body fat percentages, and higher body mass indices in both groups [55,57,61]. We also confirmed these same correlations among APOE genotyped individuals in this study (linear model, all $p < 0.05$; Table C in S2 Appendix). Further information on the THGP, OA HeLP, and participant communities are provided in the Supplementary Methods in S1 Appendix.

A Established links in urban, industrialized environments:



B Hypothesized differences in non-industrial environments:

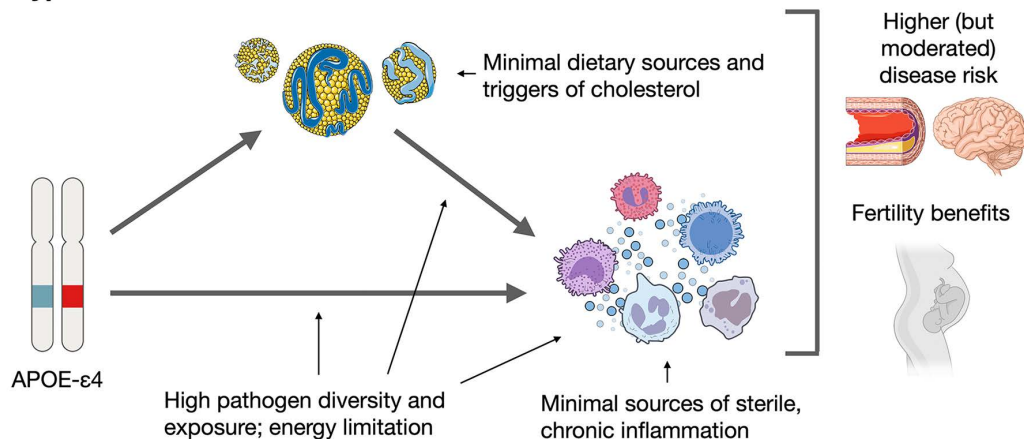


Fig 1. Conceptual model and hypothesized differences in biological relationships between non-industrial versus industrialized environments.

(A) Previous work has established that in urban, industrialized settings, 1) APOE ϵ 4 carriers exhibit elevated total and LDL cholesterol levels; 2) APOE ϵ 4 modulates innate immune function; and 3) cholesterol availability (driven by APOE genotype and other sources) impacts the formation of immune cell membranes, Toll-like receptor (TLR) signaling, and inflammasome activation. (B) In non-industrial environments characterized by limited dietary lipid availability, high pathogen burden, high physical activity, and constrained energetic resources, APOE ϵ 4 may have different biological correlates that dampen its effects on disease [17,58]. We hypothesize that, across genotypes, non-industrial lifestyles will lower total and LDL cholesterol levels and generate greater immune activation from non-sterile sources. Further, we expect that non-industrial lifestyles will reveal different APOE ϵ 4-immune and cholesterol-immune relationships relative to industrialized contexts, because both cholesterol accumulation and the reactive, APOE ϵ 4 innate immune phenotype may be advantageous in the absence of lifestyle-induced hypercholesterolemia or sterile, chronic inflammation. Adapted from [16]. Illustrations created with resources from NIH BioArt and Servier Medical Art, licensed under CC BY 4.0.

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APOE genotypes are generally in Hardy-Weinberg equilibrium

The three APOE isoforms are distinguished by just two amino acid substitutions (ϵ 2: Cys112/Cys158; ϵ 3: Cys112/Arg158; ϵ 4: Arg112/Arg158). We inferred these substitutions by genotyping the rs429358 and rs7412 single nucleotide polymorphisms (SNPs), and found that APOE isoform frequencies were consistent with previous reports [62,63]: 46.4% of Turkana ($n=513/1105$) and 35.4% of Orang Asli ($n=305/861$) participants had at least one copy of APOE ϵ 4, with only 8.1% of Turkana ($n=90/1105$) and 4.1% of Orang Asli ($n=35/861$) being ϵ 4/ ϵ 4 homozygous (Fig 3A; frequency of each haplotype in Table D in S2 Appendix).

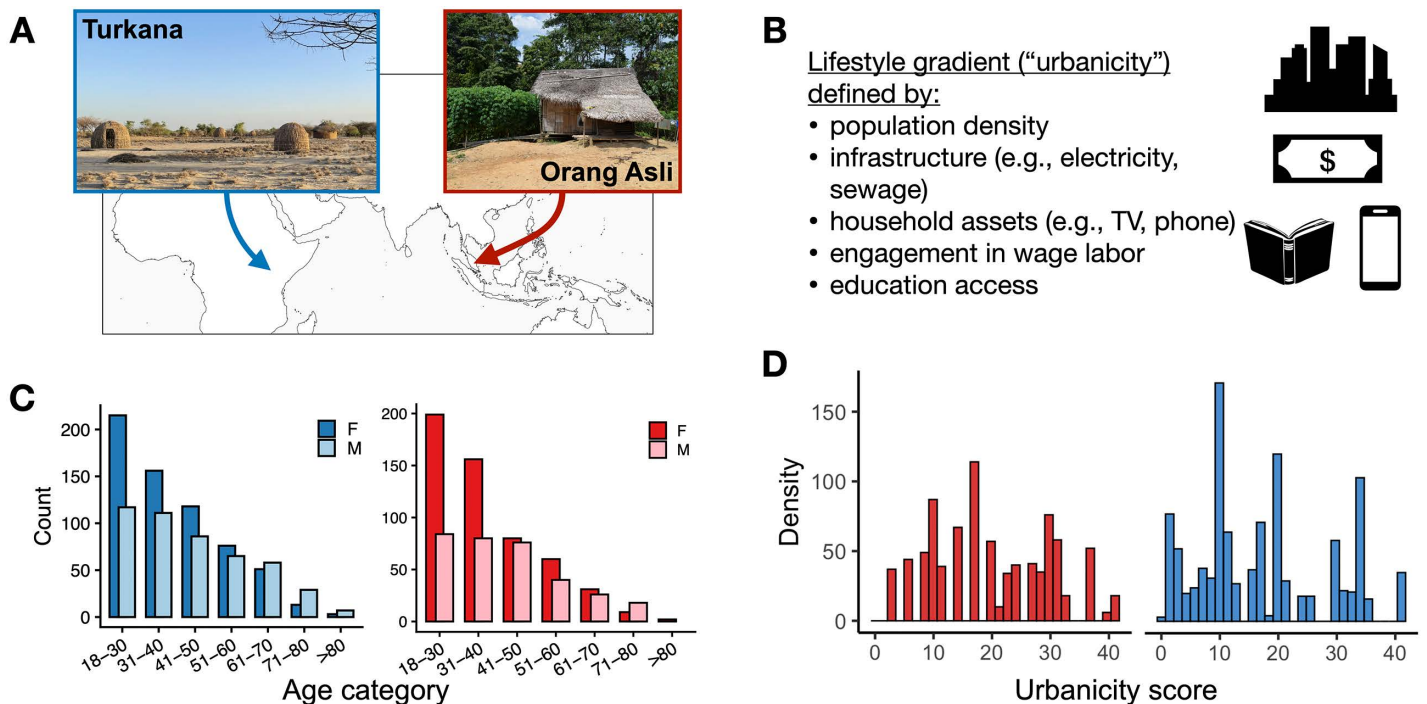


Fig 2. Study populations and lifestyle variation. (A) World map highlighting the countries where the Turkana and Orang Asli live (note Orang Asli reside specifically in Peninsular Malaysia). Pictures (which were taken by the authors) show traditional housing in non-industrial settings. Map from Free Vector Maps (<https://freevectormaps.com/world-maps/WRLD-EPS-01-0008?ref=fileID>). (B) Description of a previously validated "urbanicity" score used to quantify the continuum of non-industrial to industrialized lifestyles in a comparable way across both groups. (C) Sex and age distributions of study participants (F=female; M=male). (D) Range of urbanicity scores for the Turkana and Orang Asli sample sets. Across all panels, data from Turkana are shown in blue and data from Orang Asli are shown in red.

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Despite its associations with cardiovascular and neurodegenerative disease in industrialized societies, the frequency of APOE $\epsilon 4$ was more than three times higher than that of APOE $\epsilon 2$ in Turkana (allele frequency $\epsilon 4 = 29.8\%$, $\epsilon 2 = 9.4\%$) and 1.9 times higher in Orang Asli (allele frequency $\epsilon 4 = 22.1\%$, $\epsilon 2 = 11.1\%$). Both SNPs were in Hardy-Weinberg Equilibrium (HWE) in the Turkana (Haldane Exact Tests; rs429358, $p = 0.25$ and rs7412, $p = 0.15$). In the Orang Asli, the rs429358 SNP was in HWE, but the rs7412 was not ($p = 0.008$). However, because there is some known, slight genetic differentiation between the three Orang Asli subgroups [64], we also repeated our analyses following self-reported ancestry stratification and found that almost all subgroup-specific tests supported HWE, with the exception of rs429358 in Proto-Malay ($p = 0.03$; Table D in S2 Appendix). As the two loci that create the APOE genotype are expected to be in LD, we also treated the haplotypes as a multiallelic loci and found that all alleles were in HWE in Turkana ($p = 0.5$), Orang Asli (aggregate, $p = 0.1$), and each Orang Asli subgroup (though this test trended towards significance among the Proto-Malay; Senoi $p = 0.22$, Negrito $p = 0.24$, Proto-Malay $p = 0.06$).

APOE $\epsilon 4$ is associated with higher total and LDL cholesterol levels, as well as lower HDL cholesterol levels, across the industrialization gradient

Based on previous studies, we expected APOE $\epsilon 4$ to correlate with higher total and LDL cholesterol levels across groups and environments and found that this was the case: Turkana and Orang Asli with more APOE $\epsilon 4$ copies had higher total cholesterol (linear model, Turkana $p_{\text{FDR}} = 2.52 \times 10^{-3}$, Orang Asli $p_{\text{FDR}} = 1.18 \times 10^{-3}$), higher LDL cholesterol (Turkana $p_{\text{FDR}} = 1.30 \times 10^{-6}$, Orang Asli $p_{\text{FDR}} = 8.71 \times 10^{-7}$), and lower HDL cholesterol levels (Turkana $p_{\text{FDR}} = 4.57 \times 10^{-3}$, Orang Asli $p_{\text{FDR}} = 8.99 \times 10^{-4}$; Fig B in S1 Appendix and Table E in S2 Appendix). A somewhat unique feature of our study is that all 3

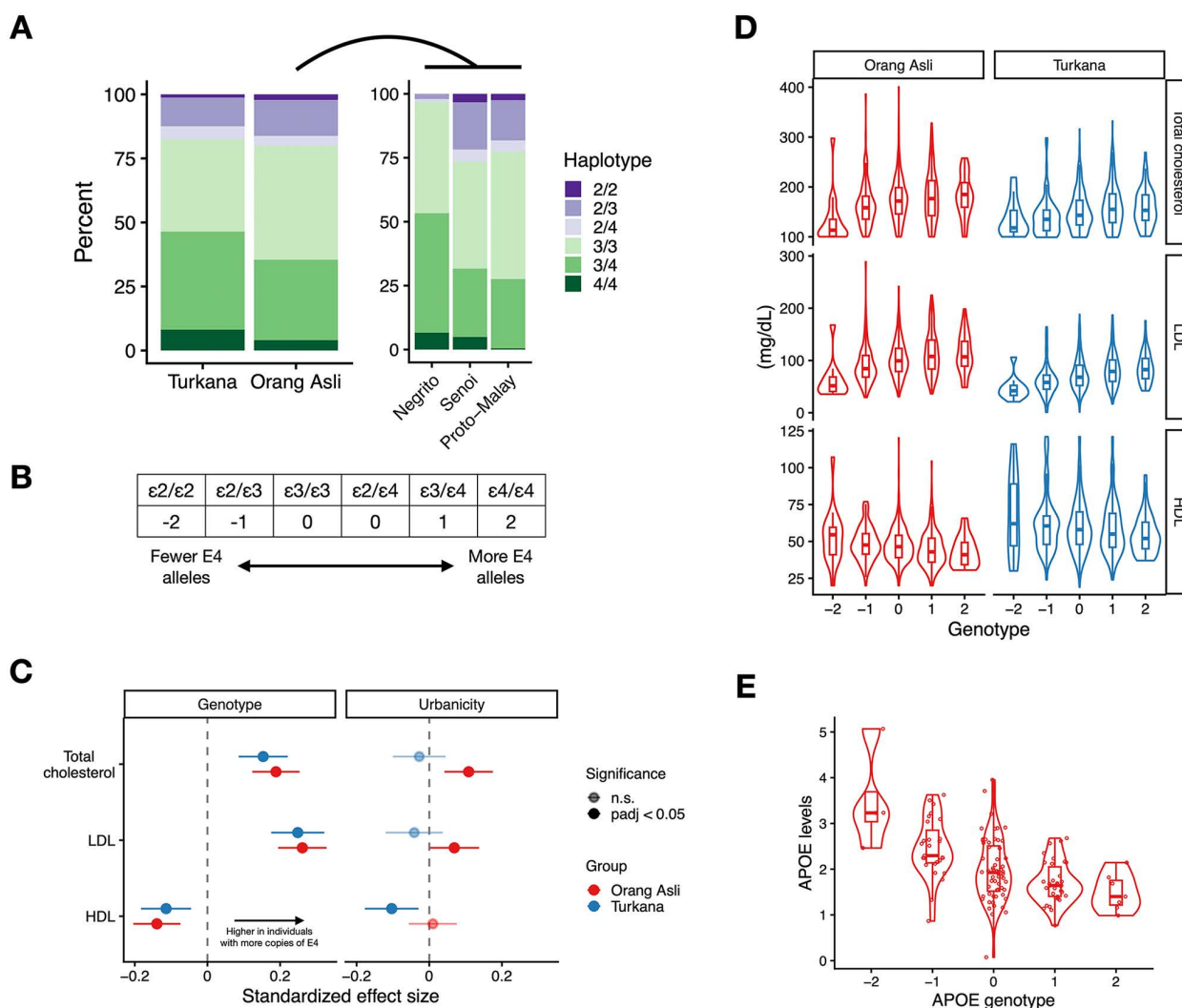


Fig 3. APOE $\epsilon 4$ is prevalent at moderate frequencies and is associated with higher blood cholesterol in Turkana and Orang Asli. (A) Frequencies of each apolipoprotein (APOE) haplotype in the Turkana and Orang Asli, with frequencies for major Orang Asli subgroups shown to the right. (B) The numeric values assigned to each APOE haplotype. (C) Forest plot of the effect of APOE (as a linear variable) on cholesterol measures from blood samples. Points represent the standardized effect size and bars represent 95% confidence intervals. Positive values indicate positive associations between cholesterol phenotypes and the linear genotype scale (i.e., individuals with more $\epsilon 4$ alleles) or living in higher urbanicity settings. (D) Cholesterol values per genotype category. Values shown are predicted from linear models of cholesterol controlling for age, sex, and urbanicity score. (E) Apolipoprotein E serum protein levels across APOE haplotypes in the Orang Asli. Values shown are predicted from linear models of protein levels controlling for age, sex, genotype, and urbanicity score.

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isoforms are represented in both groups, allowing us to compare both the APOE $\epsilon 4$ as well as the APOE $\epsilon 2$ isoform to the more common APOE $\epsilon 3$ isoform. Consistent with previous work, we found that individuals with more $\epsilon 2$ alleles had lower total cholesterol (Turkana $p_{\text{FDR}} = 8.57 \times 10^{-5}$, Orang Asli $p_{\text{FDR}} = 1.24 \times 10^{-7}$), lower LDL cholesterol (Turkana $p_{\text{FDR}} = 1.53 \times 10^{-7}$, Orang Asli $p_{\text{FDR}} = 2.26 \times 10^{-10}$), and higher HDL cholesterol levels (Turkana $p_{\text{FDR}} = 3.23 \times 10^{-2}$, Orang Asli $p_{\text{FDR}} = 3.10 \times 10^{-3}$; Fig B in [S1 Appendix](#) and Table E in [S2 Appendix](#)) relative to the other isoforms.

Because the APOE $\epsilon 4$ and APOE $\epsilon 2$ isoforms had essentially mirror opposite effects, we focused on modeling APOE genotypes as a continuous, linear variable for all remaining analyses. Specifically, we centered this measure on the APOE

$\epsilon 3/\epsilon 3$ genotype, adding one per each APOE $\epsilon 4$ copy and subtracting one per each APOE $\epsilon 2$ copy (Fig 3B). We use this approach for our main text analyses, but present parallel results using the number of APOE $\epsilon 4$ or APOE $\epsilon 2$ copies as the predictor in the Supplementary Materials.

Using the continuous approach, we confirmed our expectation that genotype linearly impacts all three cholesterol traits in both groups (all $p_{FDR} < 0.05$; Fig 3C–3D), as well as APOE serum protein levels measured for 132 Orang Asli participants (linear model, $p = 2.49 \times 10^{-9}$; Fig 3E). As expected, these effects are not lifestyle-dependent (all p_{FDR} for a genotype $\times$ lifestyle interaction > 0.1 ; Tables F–G in S2 Appendix). Nevertheless, greater urbanicity was associated with higher total ($p_{FDR} = 4.44 \times 10^{-3}$) and LDL cholesterol levels ($p_{FDR} = 0.07$, $p_{unadjusted} = 0.04$), but not HDL cholesterol levels ($p_{FDR} = 0.76$), in Orang Asli (Fig 3C). In contrast, in Turkana, greater urbanicity was associated with lower HDL cholesterol levels ($p_{FDR} = 1.85 \times 10^{-2}$) but had no effects on total and LDL cholesterol levels (Fig 3C and Tables F–G in S2 Appendix). We also investigated whether APOE genotype effects differed by sex and found highly consistent results between males and females overall, with sex-dependent effects of genotype on total cholesterol and HDL in Turkana (Fig C in S1 Appendix). Finally, sensitivity analyses revealed similar genotype effects within each of the three major Orang Asli subgroups (Fig D in S1 Appendix).

APOE isoforms have lifestyle-dependent effects on the innate immune system

Because increasingly industrialized lifestyles are associated with modifications to energy balance, physical activity, and diet in our participant populations, as well as potentially with pathogen exposure (see Discussion), we hypothesized that lifestyle should modify APOE genotype-innate immune trait associations (Fig 1). We tested this by assessing genotype $\times$ urbanicity interactions for a suite of immune biomarkers: innate immune cell counts (from a 5-part white blood cell differential), C-reactive protein (CRP) levels, plasma cytokine levels (Orang Asli only), and peripheral blood mononuclear (PBMC) gene expression levels (Orang Asli only). Several biomarkers exhibited evidence only for main effects of genotype, which generally pointed to innate immune downregulation among APOE $\epsilon 4$ carriers, consistent with previous work in industrialized contexts (Tables H–K in S2 Appendix, Fig 4A, Fig E in S1 Appendix). Specifically, in Orang Asli, continuous genotype trended toward a negative association with CRP levels (linear model, $p_{unadjusted} = 0.01$, $p_{FDR} = 0.14$; Fig 4B), indicating lower inflammation with more APOE $\epsilon 4$ alleles. Similarly, in our Orang Asli PBMC gene expression dataset ($n = 9993$ expressed genes, n samples = 745), we observed three genes significantly associated with APOE genotype at a false discovery rate of 10% (*LAMA5*, *PAIP2B*, *SIX5*). These genes are implicated in tissue development and integrity and each had higher relative expression in APOE $\epsilon 4$ carriers. We then performed gene set enrichment analyses after ranking genes based on their genotype effect sizes, and found that genes with higher expression in individuals with more APOE $\epsilon 4$ alleles were negatively enriched among pathways associated with inflammatory and innate immune activity (e.g., $p_{FDR} = 1.20 \times 10^{-13}$ for “TNF α signaling via NFKB”, $p_{FDR} = 9.73 \times 10^{-6}$ for “interferon gamma response”, $p_{FDR} = 1.47 \times 10^{-6}$ for “inflammatory response”; Fig F in S1 Appendix). The direction of these effects suggests that individuals with more APOE $\epsilon 4$ copies downregulate these processes relative to APOE $\epsilon 3$ and especially APOE $\epsilon 2$ carriers (Fig 4C–4D).

We also asked whether sex modified genotype effects on gene expression and found that while genotype effects were weakly correlated between males and females (Pearson’s $r = 0.14$, $p = 1.42 \times 10^{-47}$), formal interaction models did not reveal significant genotype $\times$ sex effects. Sensitivity analyses of genotype effects showed consistent effects of genotype within each of the three Orang Asli subgroups (full dataset compared to: Negrito [Pearson’s $r = 0.46$, $p = 2.2 \times 10^{-16}$], Senoi [$r = 0.70$, $p = 2.2 \times 10^{-16}$], Proto-Malay [$r = 0.60$, $p = 2.2 \times 10^{-16}$]).

In Orang Asli, but not Turkana, several innate immune biomarkers trended toward evidence for G $\times$ E interactions (Fig 5A). Specifically, genotype effects on the percentage ($p_{FDR} = 0.1$, $p_{unadjusted} = 1.48 \times 10^{-2}$) and total abundance of neutrophils ($p_{FDR} = 0.09$, $p_{unadjusted} = 6.41 \times 10^{-3}$) in blood, and the total amount of white blood cells ($p_{FDR} = 0.12$, $p_{unadjusted} = 2.75 \times 10^{-2}$) trended toward depending on lifestyle. These interactions consistently manifested as more copies of APOE $\epsilon 4$ being associated with higher biomarker values in rural, non-industrial contexts, but lower biomarker values in urban,

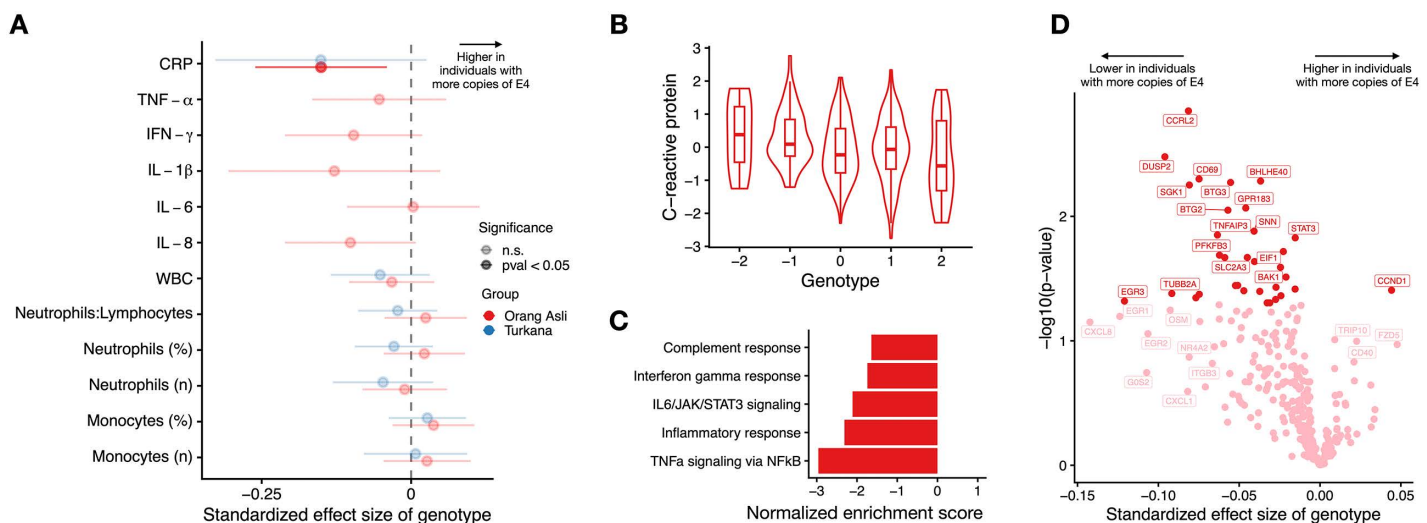


Fig 4. APOE $\epsilon 4$ is associated with lower inflammation and innate immune activity. (A) Forest plot of the effect of APOE genotype on innate immune cell count and percent, total WBC count, CRP, and innate immune biomarkers for Orang Asli (red) and Turkana (blue). **(B)** CRP values per genotype category predicted from linear models controlling for age and sex for Orang Asli. **(C)** Genes more highly expressed in individuals with more copies of $\epsilon 4$ alleles have negative enrichment of GSEA hallmark terms related to inflammation and innate immune activity in gene expression from PBMCs in Orang Asli. **(D)** Volcano plot of genes in the TNF- α signaling via NF κ B pathway. Shading indicates differentially expressed genes ($p < 0.05$).

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industrialized contexts (Fig 5B, Tables H-K in S2 Appendix). Thus, across the Orang Asli industrialization gradient, our results suggest that the previously reported immunomodulatory effects of APOE $\epsilon 4$ [33] are magnified in more urban, industrialized contexts in which they have been overwhelmingly studied.

We find very little evidence of sex-specific genotype effects on innate immune biomarkers in either population (all p for the sex $\times$ genotype > 0.05 except neutrophil to lymphocyte ratio in Turkana: $p_{\text{unadjusted}} = 4.80 \times 10^{-2}$). We also find little evidence that sex significantly modifies genotype $\times$ urbanicity effects (the only evidence observed was for IL-1B and neutrophil to lymphocyte ratio in Orang Asli), yet females consistently trend towards stronger genotype $\times$ urbanicity effects than males (Fig C in [S1 Appendix](#)). We also find that all three major subgroups of Orang Asli have consistent genotype and genotype $\times$ urbanicity effects in post hoc sensitivity analyses (Fig D in [S1 Appendix](#)).

To determine which facets of urban, industrialized environments could contribute to the GxE interactions observed in the Orang Asli, we also reran these analyses swapping out our urbanicity score for 1) body mass index (BMI), 2) an index of how much access each individual has to market-derived, processed foods [55], 3) usage of tobacco products (never versus sometimes or daily), and 4) objectively measured physical activity levels (measured as mean Euclidean Norm Minus One or ENMO from accelerometry) [61]. Each of these factors are components of the broader non-industrial to industrialized lifestyle continuum, are thought to perturb the innate immune system via sterile, chronic inflammation [47], and have been previously hypothesized as modifiers of APOE ϵ 4's effects [16,18,20–22]. None of these individual factors recapitulated GxE interactions as strongly as what we observed using our composite urbanicity score (Fig 5C). However, BMI came the closest, with trends toward GxE interactive effects on neutrophil percentage ($p_{\text{FDR}} = 0.47$, $p_{\text{unadjusted}} = 4.01 \times 10^{-2}$), and neutrophil to lymphocyte ratios ($p_{\text{FDR}} = 0.47$, $p_{\text{unadjusted}} = 7.8 \times 10^{-2}$; Fig 5D and Table L in S2 Appendix).

For comparison to previous work in the Tsimane, we also tested whether lifestyle modified links between cholesterol availability and innate immune system function (Fig 1) [16]. We found that cholesterol-immune biomarker links varied across the industrialization gradient in heterogeneous ways, but with some consistent patterns. For example, in both populations, higher total cholesterol levels were associated with lower numbers of monocytes in non-industrial

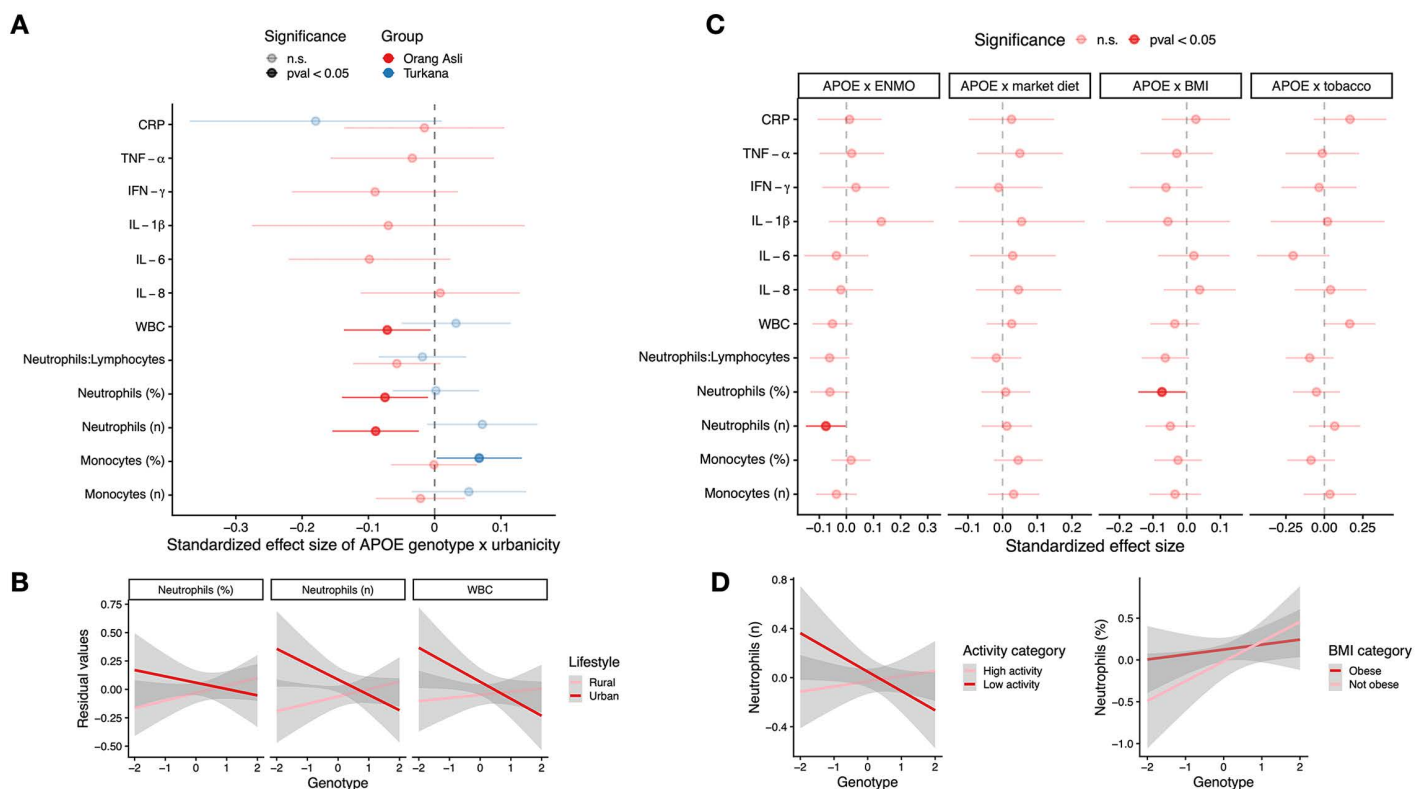


Fig 5. Some innate immune phenotypes exhibit environment x APOE genotype effects. (A) Forest plot of the effect of APOE genotype x urbanicity effects on innate immune cell count and percent, total WBC count, CRP, and innate immune biomarkers for Orang Asli (red) and Turkana (blue). (B) Innate immune biomarker values by genotype for biomarkers showing genotype by environment interactive effects in Orang Asli. Values are predicted from linear models controlling for age and sex. For visualization purposes, individuals were binned into rural and urban by urbanicity score (urban ≥ 25), though urbanicity was in practice always modeled as a continuous variable. (C) Forest plots of genotype by environment effects of several proximate, individual components of the urban environment on innate immune biomarkers (ENMO indicates physical activity, with Euclidean Norm Minus One or ENMO from accelerometry; BMI indicates body mass index). (D) Innate immune biomarker values by genotype for biomarkers showing trending genotype by mean activity level and BMI effects in Orang Asli. Values are predicted from linear models controlling for age and sex and are stratified for visualization purposes.

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environments, but higher numbers of monocytes in urban environments (Fig G in [S1 Appendix](#) and Tables M-N in [S2 Appendix](#)). The patterns we observed in non-industrial individuals are consistent with previous findings in the Tsimane, where they were interpreted as evidence that, in energy-limited contexts, high-cholesterol individuals are better able to tolerate infection [16].

APOE genotype has weak and heterogeneous effects on reproductive traits

Finally, we tested whether APOE genotype impacts female reproductive traits and whether these effects vary across the industrialization gradient (Fig 6A). We focused on age at first birth (Turkana only), age at menarche (Turkana only), age-adjusted fertility (both groups), completed fertility (both groups), and age at menopause (Orang Asli only) (Tables O-P in [S2 Appendix](#)). All reproductive information was self-reported. Overall, we found minimal evidence for genotype or GxE interaction effects, but with a few population-specific results passing our significance thresholds. First, in Turkana, we found that genotype impacted age at menarche, with more APOE $\epsilon 4$ alleles correlating with earlier reproductive maturation (Fig 6B; $n=474$, $\beta=-0.09$, $p=4.8 \times 10^{-2}$). Because this effect appeared to be driven by a later age at menarche in $\epsilon 2$

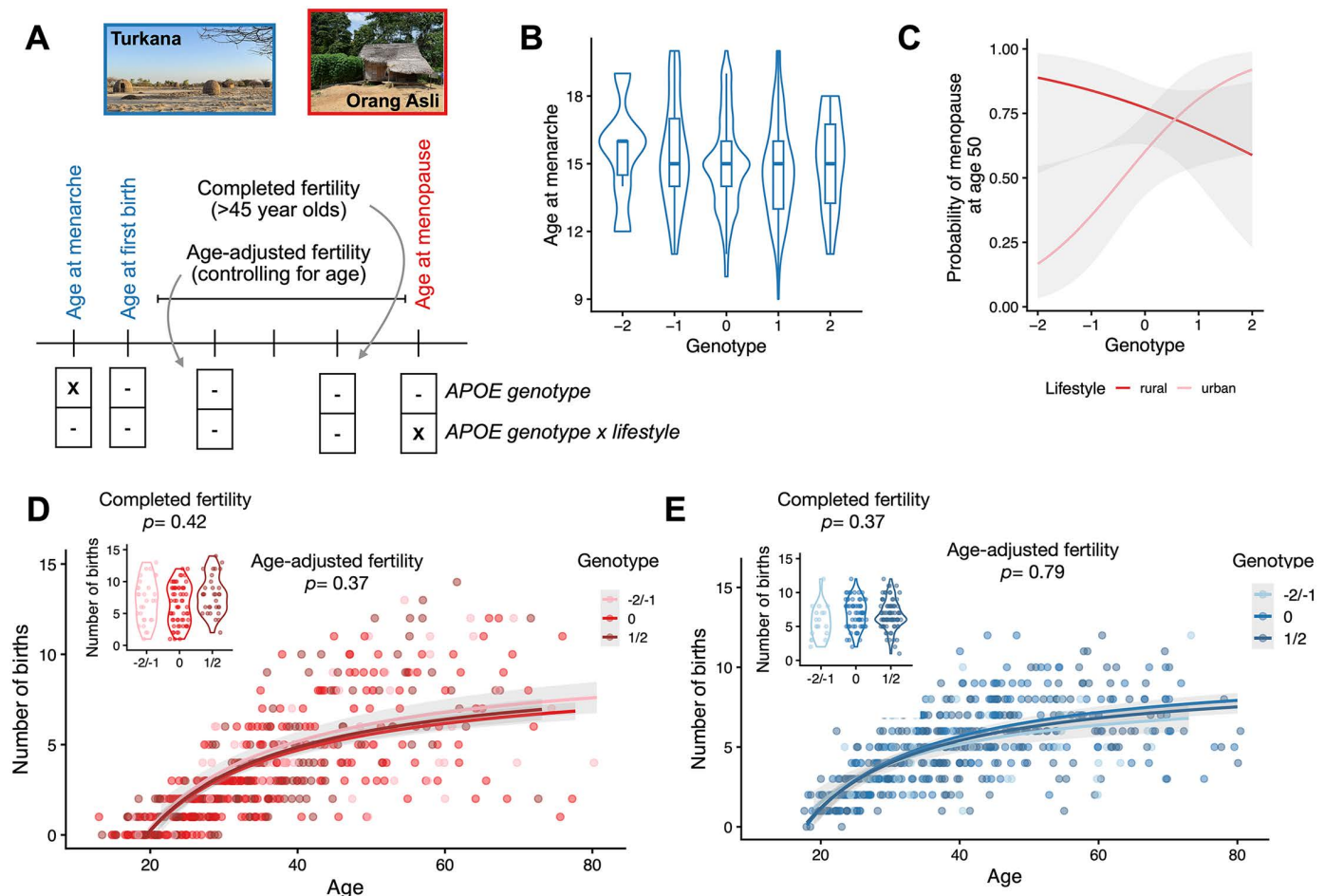


Fig 6. Select reproductive traits exhibit APOE genotype effects and genotype by lifestyle interactions. (A) Outline of key reproductive traits tested in each population with Turkana shown in blue and Orang Asli shown in red. Traits tested in both populations are labeled in black. The table shows which traits exhibited significant effects ($p < 0.05$), with the variables noted in rows. Photos were taken by the authors. (B) Turkana $\epsilon 4$ carriers report younger average age at menarche. Box/violin plots generated from raw data. (C) Probability of reporting menopause at the age of 50 among Orang Asli of various genotypes. Lines represent model predictions, shading represents the 95% confidence interval, with individuals binned into rural versus urban (although urbanicity was always modeled as a continuous variable). (D) APOE genotype is not associated with age-adjusted fertility or completed fertility (inset plot) in either Orang Asli or (E) Turkana. Lines represent linear model predictions of number of births as a function of age, fit separately for each APOE genotype group. Shading represents the 95% confidence interval and points represent the raw data.

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homozygote individuals, we performed a post hoc sensitivity analysis excluding individuals of this genotype. After exclusion, we found that the association between APOE genotype and age at menarche remained in the predicted direction and at comparable magnitude but became marginally significant at this smaller sample size ($\beta = -0.09$, $p = 0.06$). Second, in Orang Asli, we found GxE interaction effects for age at menopause: APOE $\epsilon 2$ carriers experience an earlier age of menopause in non-industrial compared to urban contexts, whereas APOE $\epsilon 4$ carriers experienced a more similar age at menopause regardless of urbanicity (Fig 6C; $n = 454$, $p = 6.02 \times 10^{-3}$). The weak, varied effects of APOE genotype on fertility outcomes that we observed were generally robust when excluding women currently on contraception (24.4% of women in OA and 18.9% of women in Turkana; Tables O-P in S2 Appendix) and to using different age cutoffs to define completed fertility (Fig H in S1 Appendix).

Discussion

Our work tests the hypothesis that the APOE $\epsilon 4$ isoform—which increases disease risk in older adults in urban, industrialized environments—has been maintained because it conferred benefits in the high-pathogen, energy-limited environments that characterized much of human evolution. While previous work has examined APOE genotype effects in non-industrial societies and has shown differential patterns relative to those reported in urban, industrialized settings [16,23–25], our design leverages data from populations spanning non-industrial to industrialized contexts to directly test for moderating effects of lifestyle. We predicted that 1) APOE genotype would have consistent effects on cholesterol traits across lifestyles, but that 2) APOE immune trait correlations would be moderated by lifestyle. We also predicted that 3) APOE genotype-fertility associations would be stronger in non-industrial relative to industrialized settings (Fig 1).

Consistent with our first prediction, APOE $\epsilon 4$ carriers exhibited higher LDL and total cholesterol and lower HDL levels than APOE $\epsilon 3$ carriers, with no evidence for GxE interactions. Notably, APOE $\epsilon 2$ displayed mirror opposite effects on cholesterol traits—both in direction and magnitude—to those of APOE $\epsilon 4$. While this is not surprising given the known biochemical differences between these isoforms [2], previous work in non-industrial contexts has not always been able to evaluate APOE $\epsilon 2$ (e.g., due to its low frequency in South America) [12]. Previous work has shown that APOE $\epsilon 2$ is protective against Alzheimer's and cardiovascular disease (relative to APOE $\epsilon 3$) in industrialized settings; however it also increases risk for several vascular and musculoskeletal diseases, suggesting complex costs and benefits [31]. Thus, evaluating the adaptive significance of APOE $\epsilon 2$ in environments relevant to human evolution is an important gap we aimed to fill. Because the Turkana and Orang Asli contain all three isoforms, we were able to construct a linear genotype variable that acknowledges and utilizes the differential impacts of APOE $\epsilon 4$ and APOE $\epsilon 2$.

APOE isoforms influence traits beyond cholesterol metabolism, with increasingly appreciated effects on innate immune function [2,13,65]. Consistent with the literature from industrialized settings [31,32], we found that APOE $\epsilon 4$ was generally associated with downregulation of innate immune activity in a baseline state (e.g., lower CRP levels, downregulation of innate immune genes). Consistent with our second prediction, we also found trends toward GxE interactions in the Orang Asli for multiple biomarkers: more APOE $\epsilon 4$ alleles trended toward associations with higher innate immune activation in rural, non-industrial contexts, but lower biomarker levels in urban, industrialized settings (i.e., urban individuals recapitulated patterns seen in industrialized contexts [31,32]). While suggestive, we cannot evaluate from these findings whether APOE $\epsilon 4$ is necessarily beneficial to immune function in non-industrial contexts. Doing so would require additional work connecting biomarkers to outcomes like pathogen clearance. However, our results point toward APOE $\epsilon 4$'s immunomodulatory phenotype being amplified by industrialization, and more generally to the idea that APOE $\epsilon 4$'s effects are environmentally modifiable—an observation relevant to the puzzle of its persistence and global patterns.

Our findings on GxE interactions raise the following key question: what components of industrialized environments are important for modifying genotype-innate immune relationships? Previous work has suggested that sterile sources of damage-associated molecular patterns (DAMPs)—including obesity, inflammatory diets, smoking, or low physical activity levels—could be relevant perturbations to innate immune and inflammatory activity [16,46–50]. Our analyses of BMI, reliance on market-derived foods, tobacco usage, and objectively measured physical activity levels point towards excess adiposity and low activity levels as relevant to genotype x industrialization interactions. We note that our composite urbanicity index showed stronger associations with immune biomarkers than any single component, indicating that cumulative DAMP exposure in urban environments likely drives GxE patterns. However, a key limitation of our study is a lack of data on pathogen exposure and load across the lifestyle gradient in both populations: specifically, the high cholesterol levels and rapidly engaged immune phenotypes associated with APOE $\epsilon 4$ are thought to be beneficial under the combination of energy limitation and high pathogen burden [17,23–26]. Thus, a major avenue for future work is to characterize pathogen load and identity alongside cholesterol, immune, and other outcomes for the same individuals.

Contrary to our third prediction, we did not observe the same robust association between APOE $\epsilon 4$ carrier status and female fertility in rural, non-industrial contexts, as was previously reported in energy-limited, high-pathogen settings in

Bolivia, Ghana, and Ecuador [23–25]. We did find evidence for earlier maturation among APOE ϵ 4 carriers and GxE effects on age at menopause, but these effects were modest. Our inability to detect genotype effects on fertility could be due to noise in self-reported data, or power that is lower than previous work in non-industrial groups (namely the Tsimane of Bolivia). Specifically, in our analyses of completed fertility ($n = 122$ and 165 in Orang Asli and Turkana, respectively), we estimate that we had $< 30\%$ power to detect differences of 0.5 children between APOE ϵ 4 carriers and non-carriers (e.g., the effect size reported for the Tsimane in [23]) (Figs 6D–6E, Fig I in S1 Appendix). While we were able to compare fertility outcomes among females who reported not currently using contraceptives, we did not have information about lifetime contraceptive usage. We also note that APOE genotype may have diverse effects on fertility correlates that we did not explore. Genotype effects on hormones or immune tolerance could impact conception probabilities: higher luteal progesterone in APOE ϵ 4 carriers may indicate higher fertility potential [66], while lower baseline inflammation in APOE ϵ 4 carriers may facilitate higher implantation success [67]. On the other hand, several studies have suggested associations between APOE ϵ 4 and recurrent pregnancy loss, but results are highly mixed [68]. Potentially most relevant, it is unclear how high the pathogen load is in Turkana and Orang Asli relative to the Tsimane or other populations with positive fertility effects. Following birth, APOE genotype could impact infant or child mortality, with previous work showing that APOE ϵ 4 carriers are less susceptible to the negative effects of enteric diseases during childhood [48,49]. Thus, while the literature has pointed toward APOE ϵ 4 carriers producing more children in non-industrial, natural fertility settings, more work is needed to uncover the precise biological path to this outcome.

Finally, observations that females in industrialized contexts have higher lifetime risk of developing Alzheimer's dementia (AD) and other APOE-associated diseases, that there are sex differences in immunological aging, and that many APOE effects are context-dependent have motivated interest in sex-specific effects [40,68–70]. Here, we find mixed evidence for sex-differences in APOE effects on circulating lipids and innate immune biomarkers, and no evidence of sex-specific genotype effects on peripheral immune gene expression. Interestingly, the immunomodulatory APOE $\times$ urbanicity effects we did uncover were all (nonsignificantly) stronger among females; this potentially suggests that vulnerability among females may be heightened in industrialized settings. We note that sex-specificity of AD in non-industrial settings is vastly under-studied and more research is needed to disentangle complex interactions between industrialization and sex on innate immune activity and disease development.

Our study has several limitations that leave scope for future work. First, we were unable to fully account for genetic relatedness and population structure in our total sample set (due to missing genome-wide genotype information for many samples, especially in the Turkana); thus, we cannot exclude the possibility that subtle population stratification confounded associations between APOE genotype and phenotypes of interest. Future studies that include fully paired genomic and phenotypic information will be important for isolating the effects of APOE. Second, we currently lack data on disease outcomes relevant to APOE (e.g., cardiovascular disease and Alzheimer's disease), which are challenging but not impossible to collect in small, subsistence-based settings. Finally, a clear priority for future work is to incorporate information on pathogen load and identity, which we hypothesize is a major driver of GxE interactions. To guide these directions, we provide a summary analysis of the major causes of childhood mortality in Kenya and Malaysia and a brief literature review of major pathogens afflicting Turkana and Orang Asli and their known APOE-associations in the Supplement. Going forward, we are also excited for studies that move beyond baseline cell states to test the hypothesis that APOE ϵ 4 carriers exhibit heightened responsiveness and pathogen clearance capacity [32,69] despite having lower baseline inflammation. For example, collecting transcriptomic data from individuals in both non-industrial and industrial settings, combined with ex vivo pathogen stimulations [70], would allow researchers to test whether GxE interactions shape the infection response. Additionally, we do not directly test for antagonistic pleiotropic effects of ϵ 4; yet benefits specific to younger individuals is a major hypothesis for ϵ 4 persistence, and future work directly testing genotype by environment interactions across the life course will be particularly valuable. Together, clarifying these myriad dynamics will not only illuminate the evolutionary drivers of APOE variation but also refine our understanding of its modern health implications.

Our results add to growing evidence that APOE ϵ 4, long considered a “detrimental” allele in industrialized settings, exerts pleiotropic and context-dependent effects that may have contributed to its maintenance. The widespread view of APOE ϵ 4 as a deleterious allele overlooks its adaptive potential in pathogen-rich, energy-limited environments, where elevated lipid levels could have fueled immune responses, reduced infection-related mortality, facilitated faster growth, and maybe even provided fertility benefits (as shown in previous work but not here) [16,23–25]. Understanding these GxE interactions is relevant to human evolution but also for informing personalized interventions [41]. For instance, APOE ϵ 4 carriers may benefit from strategies that minimize chronic inflammatory burden (e.g., prompt management of infections, low-fat diets, regular physical activity) while avoiding unnecessary lipid suppression that could compromise immune function [18]. More broadly, these findings illustrate how evolutionary perspectives can clarify apparent health “paradoxes” by situating modern disease risk within the ecological and energetic contexts in which human genotypes evolved.

Materials and methods

Ethics statement

The OA HeLP was approved by the Medical Review and Ethics Committee of the Malaysian Ministry of Health (protocol ID: NMRR-20-2214-55565), the Malaysian Department of Orang Asli Development (permit ID: JAKOA.PP.30.052 JLD 21), and the Institutional Review Board of Vanderbilt University (protocol ID: 212175). The THGP was approved by Vanderbilt University (protocol ID: 00000162) and Kenya Medical Research Institute (KEMRI/SERU/CTMDR/119/4875).

Data collection and survey overview

We used data collected from two long-term, ongoing, integrated anthropological and biomedical studies: the Orang Asli Health and Lifestyles Project (OA HeLP) and the Turkana Health and Genomics Project (THGP). OA HeLP data were collected between March 2022 and October 2024 and THGP data were collected between March 2018 and November 2023. Genotype data for this study were generated in February 2025. For both Turkana and Orang Asli participants, informed consent was collected at multiple levels: first by first describing the project to the community as a whole and seeking the permission of community leaders, and subsequently through individual-specific review of the protocol and formal written consent. Only adults (18 years and older) were included in the study. Both studies engage communities across a wide lifestyle gradient and, relevant to this study, collect information on lifestyle and reproduction (through structured surveys) combined with biospecimens and health measurements (as described in [54,59]). Specifically, our lifestyle measurements relied on questions about engagement in wage labor as well as access to mobile phones, televisions, education, electricity, and toilets/sewage (as described in [55] and the Supplementary Materials). Female Turkana participants were also asked whether they were currently pregnant, whether they were on birth control, the number of children they had given birth to, and the age at which they had their first menstrual period and first child. Among female Orang Asli participants, interviewers asked participants whether they were currently pregnant, how many children and live births they had had, whether they were currently on birth control, and whether they had undergone menopause.

APOE genotyping

To maximize the biological questions we could ask (e.g., reproductive traits in females), we prioritized female samples of reproductive age for APOE genotyping, resulting in slightly more female than male samples overall (59.5% female). To identify the APOE isoform(s) that each individual carries, we extracted DNA from whole blood samples. For both THGP and OA HeLP, whole blood was collected in EDTA tubes, immediately stored at -20°C , exported on dry ice, and subsequently stored at -80°C . DNA was extracted using the Quick-DNA Miniprep Plus or the Quick DNA/RNA extraction kits from Zymo Research and quantified using a Qubit Fluorometer. An aliquot of DNA was then shipped on dry ice to Johns Hopkins Nucleic Acid Technologies (JH-NAT) where samples were genotyped for the APOE ϵ 2, ϵ 3, and ϵ 4 alleles using

the TaqMan Allelic Discrimination system (Thermo Fisher Scientific, Carlsbad, CA). Allele determination was based on two SNPs (rs429358 and rs7412). In cases where either SNP was not confidently assigned, individuals were removed from further analyses. We tested whether rs429358 and rs7412 were in Hardy-Weinberg equilibrium using the HWExact function from the HardyWeinberg package in R [71].

Cholesterol and innate immune biomarker measurements

For all participants, EDTA-collected whole blood samples were used to measure HDL, LDL, and total cholesterol levels with a CardioCheck Plus (PTS Diagnostics), and to conduct a 5-part white blood cell differential (with a HemoCue WBC Diff System). These analyses were done immediately after blood collection so they could be available in real time and used to inform primary health care provided alongside the research study (as described in [59]). In our analyses, we focused on the following measures from the 5-part differential relevant to innate immune function: neutrophils (percent), lymphocytes (percent), monocytes (percent), neutrophils (total count), lymphocytes (total count), monocytes (total count), WBC (total count), neutrophil to lymphocyte ratio.

For a subset of Turkana participants, we also collected venous blood into a BD serum separator tube, spun the sample according to the manufacturer's instructions, froze the sample at -20°C , and used the isolated serum to later measure CRP levels via a local provider (Columbia Africa in Nairobi, Kenya) using Turbidimetric methods. For a subset of Orang Asli participants, we also collected venous blood into a BD Cell Preparation Tube (CPT), spun the sample according to the manufacturer's instructions, collected the plasma layer, and froze the plasma at -20°C . We later used these samples to measure APOE protein levels (via Targeted PRM Protein Analysis by LC-MS/MS) as well as cytokine and CRP levels (via the MSD VPLEX Human Proinflammatory Panel and CRP assay as described in [72]). These measurements were performed using aliquots sent to the Duke Proteomics and Metabolomics Core Facility and the Duke Molecular Physiology Institute Biomarkers Core Facility, respectively. The full list of measured cytokines was: TNF- α , IFN- γ , IL-10, IL-1B, IL-6, IL-12, IL-2, IL-4, and IL-8; we focus specifically on innate immune cytokines in the main text given our overall hypotheses. Finally, for a subset of Orang Asli participants, we also preserved the peripheral blood mononuclear cells isolated from the CPT tube for RNA extraction and transcriptomic analysis via mRNA-seq, following the methods detailed in the Supplementary Materials.

Statistical analyses

We performed three main sets of statistical analyses to test the predictions that: 1) APOE $\epsilon 4$ would predict higher total and LDL cholesterol levels, and industrialized lifestyles would alter 2) APOE genotype-innate immune trait association as well as 3) APOE genotype-fertility trait associations. For all figures and summary statistics, if a tested APOE $\times$ lifestyle interaction effect was not significant ($p > 0.05$), we report estimates from an additive model that does not include the interaction. Wherever we tested genotype, lifestyle, or interactive effects on multiple grouped outcomes, we corrected for multiple hypothesis testing using a Benjamini-Hochberg FDR from the p -adjust function in R (R Core Team, 2023). We use the notation $p_{\text{unadjusted}}$ to identify uncorrected p -values and p_{FDR} to identify FDR-adjusted p -values. In cases where no FDR correction was needed (e.g., because a single model was run to test a given biological hypothesis), we simply use the notation p .

Cholesterol outcomes. We asked whether APOE genotype and urbanicity predicted blood lipid levels in the Turkana and Orang Asli, as well as serum APOE protein levels in Orang Asli. For the analyses presented in the main text, we modeled APOE as a continuous variable, setting genotype $\epsilon 4/\epsilon 4$ to 2, $\epsilon 3/\epsilon 4$ to 1, $\epsilon 3/\epsilon 3$ and $\epsilon 2/\epsilon 4$ to 0, $\epsilon 3/\epsilon 2$ to -1, and $\epsilon 2/\epsilon 2$ to -2. Using linear models, we tested whether each outcome variable was predicted by age, sex, continuous APOE genotype, and urbanicity score. All variables except sex were centered and scaled. We perform stratified sensitivity analyses by sex and major Orang Asli subgroups to ask whether genotype and urbanicity effects are consistent among these groups. We also formally test for sex $\times$ genotype effects on cholesterol with models of interactive effects.

Innate immune outcomes. We used a similar linear modeling framework to ask whether the following biomarkers of the innate immune system differed across lifestyles, APOE genotypes, and their interaction (controlling for age and sex): total white blood cell count, counts and percentage of neutrophils, monocytes, and lymphocytes, and CRP in Turkana and Orang Asli, as well as innate immune cytokine and PBMC gene expression levels in Orang Asli (information on the transcriptomic analyses is provided in the Supplementary Materials). We also performed analyses stratified by sex and major Orang Asli subgroups to ask whether genotype and genotype x urbanicity effects were consistent between males and females and between the three Orang Asli subgroups. We tested for sex-differences in innate immune outcomes by modeling sex x genotype effects and, separately, sex x genotype x urbanicity effects. Measures of C-reactive protein, blood cell counts, and cytokines were $\log_2$ -transformed in both groups to reduce skewness in their distributions. After identifying several GxE interactions for innate immune outcomes in the Orang Asli, we performed follow up analyses to assess whether the following proximate factors could recapitulate patterns seen in models using the urbanicity score: 1) body mass index (BMI) calculated as weight (kg)/ height (m)², 2) an index of how much access each individual has to sugar, salt, and oil (coded as 0=none, 1=some, 2=daily and summed across all 3 items) [55], 3) usage of tobacco products (coded as 0=never versus 1=sometimes or daily), and 4) objectively measured physical activity levels from wrist worn accelerometry (operationalized as mean Euclidean Norm Minus One or ENMO, derived from the GGIR package in R [61,73]) (see Supplementary Materials). These analyses followed the same linear modeling strategy, swapping urbanicity for the variables described above. Finally, for comparison to previous work in the Tsimane [16], we tested whether lifestyle modified lipid effects on innate immune outcomes, focusing on the impacts of HDL, LDL, and cholesterol levels on the innate immune biomarkers measured in both populations (with all pairwise combinations of lipids and innate immune biomarkers explored). Here, we used linear models controlling for age, sex, main effects of the lipid of interest, main effects of urbanicity, and a lipid x urbanicity interaction.

Fertility outcomes. First, we filtered both datasets for a handful of fertility values that were improbable or internally inconsistent across outcomes (e.g., individuals that reported giving birth before reaching menarche; Supplementary Materials). Next, we used linear and generalized linear models to predict fertility traits in both groups. Specifically, we used linear models including APOE genotype, urbanicity, and a genotype x urbanicity interaction to describe age at menarche (Turkana), age at first birth (Turkana), and age at menopause (Orang Asli). We used the same predictor variables and a generalized linear modeling approach with a Poisson error structure to describe completed fertility in females 45 years or older in both populations (following [23]). We also modeled number of births in all individuals using a generalized linear model with a Poisson error structure, but including an inverse age term (1/age) as well as APOE genotype, urbanicity, and a genotype x urbanicity interaction.

Genetic relatedness and population structure. A common problem in genotype-phenotype association studies is improper control for genetic relatedness and population structure [74,75]. In other words, false positive results can occur because related individuals are more likely to have similar genotypes as well as similar phenotypes, even if genotype does not affect phenotype. To the best of our knowledge, the THGP dataset contains minimal close relatives (e.g., a single individual per household is invited to participate); this aspect of sampling design, as well as a lack of evidence for major confounding between lifestyle and population structure, has previously been tested for and described [76]. We also explore this issue further in the Supplementary Materials, reconfirming observations from previous work in this dataset. For Orang Asli, certain ethnolinguistic groups are more likely to reside in rural versus urban areas, and there is some subtle genetic differentiation between subgroups [77,78]. Therefore, we repeated all of our main analyses in a subset of Orang Asli participants using linear mixed effects models implemented in the R package EMMREML [79]. These models incorporate a random effect parameterized by a genetic relatedness matrix, which captures both close kinship and broader population structure [74]. Pairwise genetic relationships were inferred from genotypes derived from the transcriptomic data (see Supplementary Materials) and estimated in Plink [80]. These supplementary analyses agreed with the results provided in the main text and are detailed in Table Q in S2 Appendix.

We carried out all statistical analyses in the R version 4.4.2 environment.

Supporting information

S1 Appendix. A Supplementary Appendix file that includes: Supplementary Materials and Methods and Supplementary Figures. Fig A. Maps of participant villages across a lifestyle gradient in Kenya and Malaysia. Fig B. Opposing effects of APOE $\epsilon 4$ and APOE $\epsilon 2$ isoforms on cholesterol. Fig C. Sex-stratified analyses of lipids and innate immune biomarkers for each population. Fig D. Primary analyses of lipids and innate immune biomarkers for each of the three major Orang Asli subgroups. Fig E. Genotype, lifestyle, and GxE interaction effects on innate immune biomarkers. Fig F. Genotype effects on Orang Asli immune gene expression levels. Fig G. Cholesterol, lifestyle, and cholesterol x lifestyle effects on innate immune biomarkers. Fig H. Using different age cutoffs to define completed fertility does not impact conclusions. Fig I. Power to detect genotype effects on fertility. Fig J. Top causes of death among children in Kenya and Malaysia (2000–2024) from the UN Inter-agency Group for Child Mortality Estimation. Supplementary References. (DOCX)

S2 Appendix. A Supplementary Appendix file that includes: Supplementary Tables. Table A. Orang Asli participants broken down by self reported ethnolinguistic group. Table B. Turkana and Orang Asli participant demographic information. Table C. Results from linear models testing for lifestyle effects on body fat percentage and body mass index. Table D. Results from Haldane Exact tests for Hardy-Weinberg equilibrium and frequency of each haplotype per population. Table E. Results from linear models testing for genotype (number of $\epsilon 4$ or $\epsilon 2$ alleles) and urbanicity effects on cholesterol measurements. Table F. Results from linear models testing for genotype (coded linearly) and urbanicity effects on cholesterol measurements. Table G. Percent variance explained by linear model covariates testing for genotype and urbanicity effects on cholesterol measurements. Table H. Results from linear models testing for genotype (coded linearly) and urbanicity effects on immune biomarkers in Orang Asli. Table I. Results from linear models testing for genotype (number of $\epsilon 4$ allele) and urbanicity effects on immune biomarkers in Orang Asli. Table J. Results from linear models testing for genotype (coded linearly) and urbanicity effects on immune biomarkers in Turkana. Table K. Results from linear models testing for genotype (number of $\epsilon 4$ allele) and urbanicity effects on immune biomarkers in Turkana. Table L. Results from linear models testing for effects of genotype and proximate lifestyle variables on immune biomarkers in Orang Asli. Table M. Results from linear models testing for cholesterol and urbanicity effects on immune biomarkers in Orang Asli. Table N. Results from linear models testing for cholesterol and urbanicity effects on immune biomarkers in Turkana. Table O. Results from linear models testing for genotype and urbanicity effects on fertility traits in Orang Asli. Table P. Results from linear models testing for genotype and urbanicity effects on fertility traits in Turkana. Table Q. Main results (Orang Asli data only) recapitulated using linear mixed effects models. (XLSX)

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