REPÚBLICA DE ANGOLA
MINISTÉRIO DA SAÚDE
INSTITUTO NACIONAL DE INVESTIGAÇÃO EM SAÚDE
COMITÉ DE ÉTICA

CONSENTIMENTO INFORMADO, LIVRE E ESCLARECIDO PARA PARTICIPAÇÃO EM INVESTIGAÇÃO
de acordo com a Declaração de Helsínquia[1] e a Convenção de Oviedo[2]

Por favor, leia com atenção a seguinte informação. Se achar que algo está incorrecto ou que não está claro, não hesite em solicitar mais informações. Se concorda com a proposta que lhe foi feita, queira assinar este documento.

Título do estudo:

PREVALENCIA DO POLIMORFISMO DO GEN APOE (19q13.32) EM HIPERTENSOS. HUAMBO. 2020- 2023

Enquadramento:

O estudo enquadra-se no âmbito do doutoramento.

Explicação do estudo:

Estas sendo convidado para participar de um estudo sobre o polimorfismo de Apoproteína E e a hipertensão no Huambo. Os avanços na área da saúde em termos de prognóstico e tomada de decisão ocorrem através de estudos como estes, por isso a sua participação é importante. O objectivo deste estudo é saber se existe uma relação genética entre os desequilíbrios da Apolipoproteína E e a hipertensão arterial e correlacioná-las com as variáveis sócio demográficas, estilo de vida. Caso aceite o convite será necessário ser avaliado por uma ou um profissional de saúde da equipe. Sempre que quiseres poderás ter todas as informações e o seu nome não aparecerá a qualquer momento no estudo, pois identificar-te-ão com um número.

Eu _____, li ou ouvi o esclarecimento acima e compreendi para que serve o estudo e qual é o procedimento que serei submetido. A explicação que recebi esclarece os riscos e benefícios do estudo. Sei que o meu nome não será divulgado. Eu concordo em participar do estudo.

Declaro ter lido e compreendido este documento, bem como as informações verbais que me foram fornecidas pela/s pessoa/s que acima assina/m. Foi-me garantida a possibilidade de, em qualquer altura, recusar participar neste estudo sem qualquer tipo de consequências. Desta forma, aceito participar neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade e anonimato que me são dadas pelo/a investigador/a.

Nome: …….... ……..... ……
Assinatura: ……..... ……... …… Data: …… /…… /……..

[1] http://portal.arsnorte.min-saude.pt/portal/page/portal/ARSNorte/Comiss%C3%A3o%20de%20%C3%89tica/Ficheiros/Declaracao_Helsinquia_2008.pdf

[2] http://dre.pt/pdf1sdip/2001/01/002A00/00140036.pdf

SE NÃO FOR O PRÓPRIO A ASSINAR POR IDADE OU INCAPACIDADE
(se o menor tiver discernimento deve também assinar em cima, se consentir)

NOME: ……

BI/CD Nº: ..... DATA OU VALIDADE ….. /…. / ….....

GRAU DE PARENTESCO OU TIPO DE REPRESENTAÇÃO: .....

ASSINATURA ……

ESTE DOCUMENTO É COMPOSTO DE … PÁGINA/S E FEITO EM DUPLICADO:
UMA VIA PARA O/A INVESTIGADOR/A, OUTRA PARA A PESSOA QUE CONSENTE

Assinatura do pesquisador responsável: (+244) 923237696

REPÚBLICA DE ANGOLA
MINISTÉRIO DA SAÚDE
COMITÉ DE ÉTICA

(CEMS)

PARECER N.º 031 /C.E.M.S/2023

TÍTULO DO PROJECTO: PREVALÊNCIA DO POLIMORFISMO DO GEN APOE (19q13.32) EM HIPERTENSOS HUAMBO. 2020-2025.

INVESTIGADOR RESPONSÁVEL: JOB CHIVANGULULA PAKISI

PARECER DO C.E.M.S:

Considerando o protocolo pouco elaborado, e sem uma descrição detalhada, é necessário que o investigador inclua informações sobre como será feita a colheita, o processamento e tratamento dos dados e amostras, as ferramentas de proteção e confidencialidade de dados. Entretanto, é ainda necessário esclarecer onde será feito o estudo e se o material será transportado;

O Comité emitiu o parecer de **APROVADO PROVISÓRIO** para o projecto "Avaliar a literacia comunicativa em saúde nos serviços de saúde".

Portanto, deverá ressubmeter o protocolo, com as recomendações que lhe foram dadas.

Segundo normas do C.E.M.S para os protocolos aprovados, deve ser enviado o relatório de acompanhamento e de término ao Secretariado, conforme modelo disponível na página http: //www.inis.gov.ao/comite-de-etica/.

Luanda, 14 De Agosto de 2023.

COMITÉ DE ÉTICA DO MINISTÉRIO DA SAÚDE
CEMS
INIS

Joana Paixão
(Ph.D., MSc., BSc.)
Coordenadora Interina do C.E.M.S

Rua Amílcar Cabral Nrº. 96, à frente do Banco de Urgências do Hospital Josina Machel, Maianga – Luanda, Angola. **Telefone:** +244 924 390 307 **e-mail:** comitedeetica@inis.gov.ao e comitedeetica91@gmail.com

Apolipoprotein E (APOE) Allele Frequencies and Genotypic Distribution in Huambo, Angola

Running title: Apolipoprotein E allele frequencies in Angola

Author's list

Job Pakisi, MSc[1,2,3], Vicente Martín-Sánchez[2,3,4], MD, PhD[2,3,4], Cruz S. Sebastião, PhD[5,6,7], Victor Moreno, PhD[8,9,10,11], E. Bayón-Darkistade, PhD[2,3]

Author's affiliations

[1]Faculdade de Medicina (FM), Universidade José Eduardo dos Santos (UJES), Huambo, Angola;

[2]Universidade de León, León, España;

[3]Instituto de Biomedicina, León, España

[4]Ciberersp, España

[5]Centro de Investigação em Saúde de Angola (CISA)|Instituto Nacional de Investigação em Saúde (INIS), Luanda, Angola;

[6]Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal;

[7]Centro Nacional de Investigação Científica (CNIC), Luanda, Angola.

[8]Catalan Institute of Oncology, Barcelona, España

[9]Institut d'Investigacio Biomedica de Bellvitge, Barcelona, España

[10]Universitat Autònoma de Barcelona, España

[11]University of Barcelona, Barcelona, España

*Correspondences

[1,2,3]Job Pakisi, MSc. Email: jobpakisi19@gmail.com

ABSTRACT

Background: Non-communicable diseases (NCDs) are a leading cause of death globally, particularly in low- and middle-income countries undergoing rapid epidemiological transition. Apolipoprotein E (ApoE) polymorphisms have been implicated in modulating risk for cardiovascular and neurodegenerative diseases. This study characterizes the distribution of APOE alleles and genotypes in an Angolan population sample and explores associations with demographic variables. Methods: A cross-sectional study was conducted in Huambo Province, Angola, with 200 unrelated adults aged 40 –70 years. Genotyping for ApoE2, ApoE3, and ApoE4 was performed via real-time PCR. Allelic and genotypic frequencies were calculated and tested for Hardy-Weinberg equilibrium. Associations with sex, age (≤54 vs >54 years), and urban vs rural residence were analyzed. Results: ApoE3 was the most frequent allele (63%), followed by ApoE4 (24.8%) and ApoE2 (12.2%). The ApoE3 homozygous genotype was prevalent (44%), with ApoE3/E4 (19%) and ApoE2/E3 (18%) also common. Significant Hardy-Weinberg disequilibrium was observed due to excess ApoE4 homozygotes and deficit of certain heterozygotes, consistent with population structuring and endogamy. ApoE4 prevalence was higher in urban residents and older individuals, while ApoE3 and ApoE2 were more common in rural areas and younger participants. Interpretation: Our results reveal notable genetic heterogeneity and highlight the epidemiological and evolutionary importance of ApoE4 in Angola. The findings underscore the need for integrating population genomics into public health strategies targeting NCD prevention, especially in rapidly urbanizing African contexts.

KEYWORDS: APOE, Allele frequency, Angola, Non-communicable diseases.

1 INTRODUCTION

Non-communicable diseases (NCDs) account for three out of every four deaths worldwide, and three out of four of these deaths occur in low- and middle-income countries (1). Demographic changes and lifestyle transitions underlie the high prevalence of these diseases and their concerning rapid increase in emerging countries (2). Additionally, interactions between genetic and environmental factors play a crucial role in the incidence and course of these diseases and must be considered when designing prevention and control strategies (3,4).

Apolipoprotein E (ApoE) contributes to the modulation of immune and inflammatory responses, and its different polymorphisms can have differential effects on some of the most relevant NCDs such as cardiovascular, autoimmune, and neurodegenerative diseases (5,6). The clinical relevance of ApoE alleles lies in their pleiotropic impact: the ε3 allele is usually associated with a balanced lipid profile, ε2 with some cardiovascular protection in heterozygotes (though with a risk of dyslipidemia in homozygotes), and ε4 with a pro-atherogenic phenotype characterized by increased LDL cholesterol, decreased HDL cholesterol, and a greater predisposition to both atherosclerosis and cognitive decline including Alzheimer's disease (7).

The global distribution of ApoE alleles shows marked geographic and ethnic gradients (8). Worldwide, the ε3 allele, with prevalences exceeding 70%, is the most frequent and considered the wild-type allele. The ε4 allele shows variable frequency and can reach up to 30% in some African populations. The ε2 allele is the least common, generally not exceeding 10%. These differences reflect patterns of positive selection, environmental pressures, and human migrations (8).

Angola, a Southwestern African country characterized by remarkable ethnic heterogeneity and a history marked by migrations and admixture (9), constitutes a unique setting to analyze the genetic variability of APOE and its relationship with emerging chronic diseases, which cause one in four deaths in the country (10). This study aims to characterize the frequencies of APOE polymorphisms in Angola and their relationship with demographic variables.

2 MATERIAL AND METHODS

2.1 Study design and setting

A cross-sectional study was conducted in Huambo Province, Angola, including a convenience sample of 200 unrelated adults (100 men and 100 women), half residing in urban areas and half in rural. Participants were aged

between 40 and 70 years (mean 55 ± 9 years; median 54). Exclusion criteria included major neurological diseases, known high risk of severe cardiovascular disease, pregnancy, and HIV infection. The study was approved by the Scientific Council of the Faculty of Medicine of Huambo (Deliberation No. 003/CC/23) and the Ethics Committee of the Angolan Ministry of Health (031/C.E.M.S./2023).

2.2 Sample collection and processing

Peripheral blood was collected in EDTA tubes. Genomic DNA was extracted using QIAamp DNA Mini Kit (QIAgen). ApoE genotyping for alleles ApoE2, ApoE3, and ApoE4 was performed by real-time PCR (StepOne 7500, Applied Biosystems) with SYBR Green and specific primers. Samples were analyzed in duplicate, with positive and negative controls; a Ct value < 25 indicated allele positivity. Demographic data collected included sex, age (categorized as ≤54 or >54 years), and place of residence (urban or rural). Allelic and genotypic frequencies were calculated from PCR results (10)

2.3 Statistical analysis

Descriptive statistics were used to calculate allelic and genotypic frequencies, expressed with 95% confidence intervals (Clopper-Pearson method). Hardy-Weinberg equilibrium was assessed with chi-square tests ($\alpha = 0.05$). The inbreeding coefficient (F-IS) was computed as F-IS = (He - Ho) / He, where He is expected heterozygosity and Ho is observed heterozygosity. Allelic frequencies were compared using contingency chi-square tests; genotypic frequencies were compared using Fisher's exact test.

3 RESULTS

The ApoE3 allele had the highest frequency, present in approximately two-thirds of participants, followed by ApoE4 (24.8%) and ApoE2 (12.2%) (Table 1, Figure 1). Genotypic analysis revealed 62.5% homozygotes and 37.5% heterozygotes. The most frequent genotype was ApoE3 homozygous (44.0%; 95% CI: 37.6–50.4), followed by ApoE3/E4 (19.0%; 95% CI: 13.2–24.8), ApoE2/E3 (18.0%; 95% CI: 12.3–23.7), and ApoE4/ε4 (15.0%; 95% CI: 10.1–19.9). ApoE2/E2 and ApoE2/ε4 genotypes were least frequent (3.5% and 0.5%, respectively). Hardy-Weinberg equilibrium testing showed significant deviation ($\chi^2 = 51.73$; df = 3; $p < 0.001$), driven by an excess of ApoE4 homozygotes and a deficit of ApoE2/ε4 and ApoE3/ε4 heterozygotes. The inbreeding coefficient (F_IS = 0.295) indicated a substantial deficiency of heterozygotes. This pattern persisted across sexes, urban/rural residence, and age groups. Significant differences were observed in allelic and genotypic distributions by residence and age but not sex. ApoE3 was more prevalent in rural areas (72.0% vs

53.0% urban), while ApoE4 was more prevalent in urban residents (34.0% vs 15.5% rural). Similarly, ApoE3/3 homozygotes predominated in rural (59.0%) versus urban (29.0%) areas, with ApoE3/4 heterozygotes more common in urban settings (28.0% vs 10.0%). Age comparisons showed higher ApoE4 frequency in older participants (>54 years: 34.3% vs ≤54 years: 15.4%) and higher ApoE2 in younger individuals (16.8% vs 8.6%), with corresponding genotypic differences (E2/3 and E4/4).

4 DISCUSSION

This is the first study to describe the allelic and genotypic prevalences of apolipoprotein E in the Angolan population. Our findings highlight the remarkable genetic and phenotypic heterogeneity present in the African continent, as well as the coexistence of historical adaptive advantages with emerging risks stemming from changes in lifestyle. The ε4 allele, which confers an advantage in environments characterized by high infectious burden and nutritional limitations, is significantly associated with adverse impacts in urbanized settings with Western lifestyles, marked sedentarism, and high-calorie diets. This represents a critical challenge for public health strategies aimed at preventing and managing emerging diseases, particularly cardiovascular and neurodegenerative disorders.

In our sample, the ApoE3 allele was the most prevalent, with an approximate frequency of 63%, a figure that falls within the commonly reported range in global and regional studies, where ApoE3 typically varies from 55% to 90% across different human populations, showing closer similarity to prevalences reported for other sub-Saharan groups (8,11). This prevalence reaffirms the ancestral and dominant nature of the ApoE3 allele in most populations, including African and Afro-descendant groups, strengthening the validity and consistency of our results in the population genetics context.

The frequency of the ε4 allele shows considerable worldwide variation, ranging from 0% in some Indian populations to nearly 50% in certain Brazilian or African tribes (12). In our case, the observed value of 24.8% represents an intermediate level within the African spectrum. While North African regions, such as Morocco and Tunisia, report low frequencies (<10%), other West African countries (such as Senegal: 3%) also exhibit considerably lower levels. In contrast, countries like Uganda (25%), Rwanda (24%), South Africa (25.4%), Nigeria (30%), Sudan (29%), and particularly Khoisan groups (37%) and Central African pygmies (41%) present high prevalences (13–15). This finding underscores the genetic heterogeneity within the African continent and emphasizes the uniqueness of the Angolan population structure.

The high prevalence of the ε4 allele, especially in its homozygous form, partly explains the significant genotype disequilibrium observed. The locally elevated persistence of ε4 may be modulated by adaptive factors such as infection resistance or reproductive advantages, as proposed for other African populations (14,16), and also suggests population structure and endogamy consistent with localized marital practices in Angola. This phenomenon, widely documented in African studies, highlights the need to account for population substructure to avoid bias in genetic association analyses and phenotypic risk interpretation (17), also explaining the high inbreeding coefficient observed.

This dynamic balance of risks and benefits underscores the importance of a personalized approach in medicine and public health for countries undergoing epidemiological transition.

The epidemiological relevance of the ε4 allele lies in its well-established association with increased risk of cardiovascular and neurodegenerative diseases in Western lifestyle contexts. However, in Africa, phenotypic plasticity attributable to environmental, infectious, and behavioral factors is observed (18–20). As documented by Masemola et al. in South Africa, rural-urban transitions and lifestyle changes may precipitate future cardiovascular epidemics in ε4 carriers, emphasizing the urgency of genetic-environmental surveillance in Angola to anticipate disease burden evolution (21).

Analyzing the biomedical impact of the ε4 allele, several studies have confirmed its relationship with elevated total cholesterol, LDL-c concentrations, and reduced HDL-c, findings consistent with extensive international evidence (22–25). This positions ε4 as a potential driver of the epidemiological transition in developing countries, accelerating the prevalence of non-communicable diseases (NCDs). On the other hand, the ε2 allele, while usually associated with a favorable lipid profile in heterozygotes, can predispose to severe dyslipidemias (type III) in the presence of factors such as diabetes and excessive alcohol intake, reinforcing the multifactorial nature of clinical risk (26–28).

Regarding urban-rural differences, the predominance of ApoE3 in rural areas (72%) contrasts with the higher frequency of ApoE4 in urban environments (34%), probably due to internal migrations, inequalities in healthcare access, and lifestyle variations. This pattern suggests that urban exposure may amplify cardiovascular and neurodegenerative risk for ε4 carriers, in line with global findings (29).

Age distribution is also relevant: the higher prevalence of ApoE4 among older individuals (≥54 years) and predominance of ApoE2 in younger cohorts raise questions about natural selection and cohort dynamics. While

ApoE4 has traditionally been linked to reduced longevity (30), Lane et al. demonstrated that its impact on mortality varies significantly according to ethnic and geographic context, potentially exhibiting neutral effects in African and Afro-descendant groups (17). A more recent study by Vivian et al. found no association between ApoE4 genotypes and mortality in elderly individuals ≥80 years; however, classical cardiovascular factors such as smokings and diabetes increased mortality risk, whereas physical activity and elevated systolic blood pressure reduced it (31). Therefore, it is essential to deepen longitudinal studies incorporating environmental and clinical variables to clarify these effects (32).

In conclusion, these findings emphasize the urgency of public health policies integrating population genomics as a tool for risk assessment and healthcare planning, especially in rapidly changing urban African contexts. Targeted preventive interventions should be prioritized, as recommended by Masemola et al., to mitigate the rise of NCDs in genetically susceptible groups and to test new personalized medicine strategies. Future research should expand sample sizes, incorporate additional genetic markers and clinical-metabolic variables, and conduct multicenter and trans-ethnic meta-analyses following models such as Marini et al., which enable a comprehensive understanding of genetic, environmental, and population health interactions. This study significantly contributes to describing the ApoE genomic profile in Angola, demonstrating the predominance of the ApoE3 allele, but confirming the sustained epidemiological and evolutionary importance of ApoE4, the presence of endogamy and population structuring, and the potential impact of these factors on African medicine and public health.

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DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available on request from the corresponding author.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

FINDING

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization: JP and ED. Investigation: JP, VM_S, CSS, VM_S, and EB-D. Methodology: JP, VM_S, and EB-D. Validation: JP, and EB-D. Data curation: JP, VM_S, CSS, and ED. Formal analysis: JP, VM_S, and ED. Data collection: JP. Supervision: ED. Writing - original draft: JP, VM_S, CSS, VM and ED. Writing – review & editing: JP, VM_S, CSS, VM, and ED. All authors approved the final manuscript for publication.

ORCID

Job Pakisi – 0009-0006-9343-1126

Vicente Martín-Sánchez - 0000-0003-0552-2804

Cruz S. Sebastião – 0000-0003-1232-0119

Victor Moreno – 0000-00022818-5487

E. Bayón-Darkistade – 0000-0002-0562-8281

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Table 1. Distribution of genotypic and allelic variants according to various variables.

Genotypes and Alleles	1.Urban $			2.Rural $			0.Male *			1.Female *			0.Age&			1.Age&			TOTAL			
	%	IC95%		%	IC95%		%	IC95%		%	IC95%		%	IC95%		%	IC95%		N	%	IC95%	
E2/E2	3.0	0.6	8.4	4.0	1.1	9.8	3.0	0.6	8.4	4.0	1.1	9.8	4.0	1.1	9.9	3.0	0.6	8.5	7	3.5	0.9	6.1
E2/E3	20.0	12.6	29.3	16.0	9.4	24.8	19.0	11.6	28.2	17.0	10.0	26.2	**25.0**	17.1	34.1	**11.0**	5.5	18.7	36	18.0	12.3	23.7
E2/E4	0.0	0.0	3.6	1.0	0.0	5.6	0.0	0.0	3.6	1.0	0.0	5.6	1.0	0.0	5.4	0.0	0.0	3.6	1	0.5	0.0	1.5
E3/E3	**29.0**	20.4	39.1	**59.0**	48.6	67.0	45.0	35.1	55.1	43.0	33.0	53.2	49.0	39.1	57.9	39.0	29.2	48.6	88	44.0	37.6	50,4
E3/E4	**28.0**	19.6	37.7	**10.0**	4.9	18.0	14.0	8.0	22.3	24.0	15.5	33.8	14.0	8.0	22.1	24.0	15.9	33.3	38	19.0	13.2	24.8
E4/E4	20.0	12.6	29.3	10.0	4.9	18.0	19.0	11.6	28,2	11.0	5.6	18.7	**8.0**	3.4	15.1	**22.0**	13.8	31.6	30	15.0	10.1	19.9
E2	13.0	8.3	17.7	12.5	7.9	17.1	12.5	7.9	17.1	13.0	8.3	17.7	**16.8**	11.7	21.9	**8.6**	4.7	12.4	51	12.8	9.6	16.0
E3	**53.0**	46.1	59.9	**72.0**	65.8	78.2	61.5	54.8	68.2	63.5	56.9	70.1	67.8	61.4	74.2	57.1	50.2	64.0	250	62.5	57.8	67.3
E4	**34.0**	27.5	40.5	**15.5**	10.5	20.5	26.0	19.9	32.0	23.5	17.7	29.3	**15.4**	10.3	20.3	**34.3**	27.7	40.9	99	24.7	20.6	28.9
Genotypes	**$ p-value = 0.0001**						* p-value = 0.2813						**& p-value = 0.0025**									
Alleles	**$ p-value <0,0001**						* p-value = 0.85						**& p-value <0,0001**									

Figure 1. Distribution of genotypes and allelic frequencies and their 95% confidence intervals.

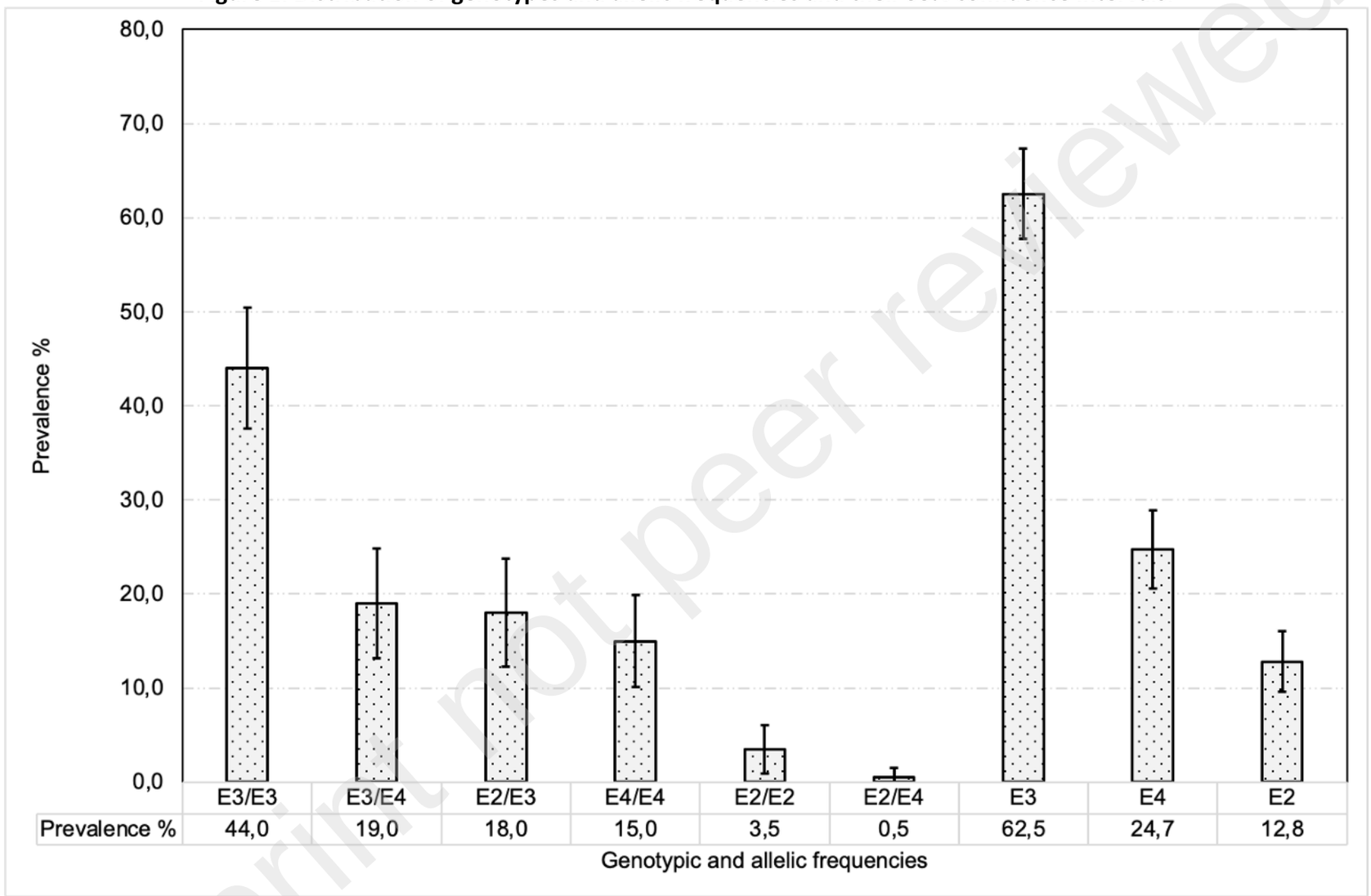
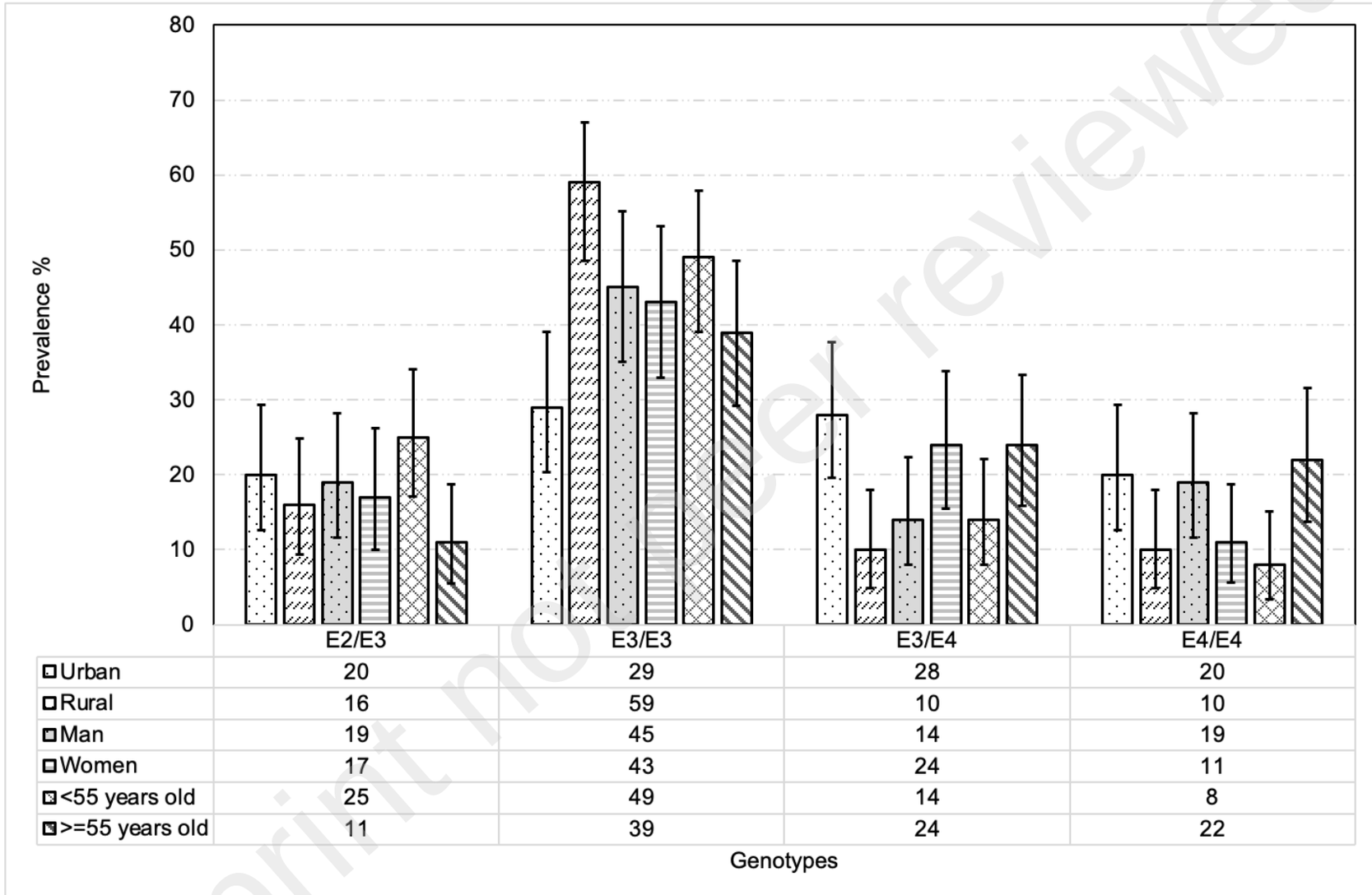


Figure 2. Distribution of genotypic variables according to the different categories analyzed.



	E2/E3	E3/E3	E3/E4	E4/E4
Urban	20	29	28	20
Rural	16	59	10	10
Man	19	45	14	19
Women	17	43	24	11
<55 years old	25	49	14	8
>=55 years old	11	39	24	22

Genotypes

Dear Editor, Dr. Zoë Mullan,

I am submitting for your consideration the manuscript entitled “Apolipoprotein E (APOE) Allele Frequencies and Genotypic Distribution in Huambo, Angola.” This is the first study to characterize APOE allele and genotype distributions in an Angolan population from Huambo Province, highlighting their association with demographic factors such as age, sex, and urban/rural residence.

Our findings show significant genetic heterogeneity, especially a higher prevalence of the ApoE4 allele in urban and older individuals. This genetic insight is crucial for informing public health strategies that integrate genomics to tackle the rising burden of non-communicable diseases in rapidly urbanizing African settings.

We believe this manuscript will interest The Lancet Global Health readership for its implications in global health policy regarding vulnerable populations in transition.

The study was conducted with full ethical approval and there are no conflicts of interest to declare.

Thank you for your consideration. I am available for any further information you may require.

Sincerely,

Job Chivangulula Pakisi, MSc.
José Eduardo dos Santos University
University of Leon. Email: Jobpaksi19@gmail.com, Jpakis00@estudiantes.unileon.es
7 August 2025

Assinatura electrónica
Job Chivangulula Pakisi
Data: 7/08/2025