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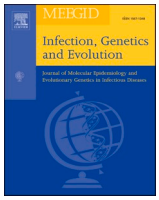

Polymorphisms in the human angiotensin converting enzyme gene (*ACE*) linked to susceptibility of COVID-19 and malaria infections in the Ghanaian population

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ABSTRACT

Genetic variations in the human angiotensin converting enzyme gene (*ACE*) influence *ACE* enzyme expression levels in humans and subsequently influence both communicable and non-communicable disease outcomes. More recently, polymorphisms in this gene have been linked to susceptibility and outcomes of infectious diseases such as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and malaria infections. This study is the first to investigate the genetic diversity of *ACE* and *ACE2* polymorphisms in the Ghanaian population. Archived filter blood blot samples from malaria patients aged ≤9 years were used. Molecular analysis for the detection of *ACE* rs4646994 (I/D), *ACE2* rs2106809 (C/T) and rs2285666 (G/A) alleles as well as *ACE2* exons 1–4 polymorphisms was conducted on 300 samples. The D allele (54%,162/300) was the most dominant polymorphism observed in the *ACE* rs4646994 gene whilst the G (68%, 204/300) and T alleles (59.3%,178/300) were the most frequent *ACE2* rs2285666 and rs2106809 polymorphisms observed. For the 300 samples sequenced for *ACE2* exons 1–4, analyses were done on 268, 282 and 137 quality sequences for exons 1, 2 and 3–4 respectively. For exon 1, the mutation D38N (2.2%; 6/268) was the most prevalent. The S19P and E37K mutations previously reported to influence COVID-19 infections were observed at low frequencies (0.4%, 1/268 each). No mutations were observed in exon 2. The N121K/T variants were the most seen in exons 3–4 at frequencies of 5.1% (K121, 7/137) and 2.9% (T121, 4/137) respectively. Most of the variants observed in the exons were novel compared to those reported in other populations in the world. This is the first study to investigate the genetic diversity of *ACE* and *ACE2* genes in Ghanaians. The observation of novel mutations in the *ACE2* gene is suggesting selection pressure. The importance of the mutations for communicable and non-communicable diseases (malaria and COVID-19) are further discussed.

1. Introduction

The effect of genetic diversity in genes encoding proteins of importance for human health and susceptibility to both communicable and non-communicable diseases has in the recent past become crucial for investigation. One of such proteins/enzymes is the angiotensin converting enzyme (ACE) and its homologue the ACE2 (Donoghue et al., 2000). The genes *ACE* and *ACE2*, sharing 42% of amino acids, code for two carboxypeptidases which are major components of the human renin-angiotensin system (RAS) (Donoghue et al., 2000). The enzymes ACE and ACE2 have been reported to play opposing roles in the RAS. ACE converts angiotensin I (Ang I) to angiotensin II (Ang II), a hormone that constricts blood vessels whilst ACE2 converts Ang II to angiotensin (1–7), a hormone that has vasodilatory effects. The opposing effect between the two enzymes, ACE and ACE2, is as the expression of the former decreases, the latter increases (Çelik et al., 2021; Delanghe et al., 2020).

ACE2 is expressed in all tissues, with the small intestine, kidneys, testicles, thyroid, heart and adipose tissue expressing it at the highest levels, followed by the liver, lungs, colon, bladder and adrenal gland (Aguiar et al., 2020; Li et al., 2020). Human ACE2, encoded by the *ACE2* gene, is located on the X chromosome (Xp22) in a region where genes are known to escape X-inactivation, leading to differences in phenotypes between males and females (Burrell et al., 2013; Carrel and Willard,

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2005; Chen et al., 2021). ACE2 has diverse functions including the regulation of blood pressure through the dilation of blood vessels (De et al., 2021a; Stewart et al., 2008) and the absorption of amino acids in the kidney and gut (Kuba et al., 2010). In the COVID-19 pandemic, it has been observed that ACE2 serves as a receptor for the entry of the severe acute respiratory syndrome coronavirus 2 virus (SARS-COV 2) into host cells (Gómez et al., 2020; Hoffmann et al., 2020; Walls et al., 2020; Yan et al., 2020).

Genetic variations in the *ACE2* gene are thought to be a major factor in determining ACE2 activity (Çelik et al., 2021; Burrell et al., 2013). These variations have gender-specific effects, with males reportedly having significantly higher ACE2 expression than females (Gagliardi et al., 2020; Zhao et al., 2020) which is based on the idea that males are hemizygous for these variants by possessing one X chromosome and females are homozygous for these variants (Chen et al., 2021). Also, previous studies have shown that different genetic variants of *ACE2* have been observed to influence varying ACE2 expression levels in humans (De et al., 2021b; Hou et al., 2020; Liu et al., 2016; Clarke and Turner, 2012; Dhangadamajhi et al., 2010; Fan et al., 2007; Rice et al., 2006; Rigat et al., 1990). These variations have also been linked to the pathogenesis of several communicable and non-communicable diseases such as diabetes, hypertension, malaria and most notably the coronavirus disease (COVID-19) (Bosso et al., 2020).

Reports postulate that single nucleotide polymorphisms (SNPs) in the *ACE2* gene may impact the level of expression of the ACE2 protein and the ability of the SARS-CoV-2 virus to bind to host cells which in turn, may affect susceptibility to the disease (Möhlendick et al., 2021; Çelik et al., 2021; Darbani, 2020; Devaux et al., 2020; Gemmati et al., 2020; Hou et al., 2020). A number of *ACE2* genetic variants have been identified and these, S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P and H378R, are thought to increase susceptibility to SARS-CoV-2 infections whilst others, K31R, N33I, H34R, E35K, E37K, D38V, Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, G352V, D355N, Q388L and D509Y, protect against the infection (Suryamohan et al., 2021). Despite these reported variants, the rs2106809 T and rs2285666 G alleles in introns 1 and 3 respectively have been particularly noteworthy as they have been linked to severe clinical outcomes of SARS-CoV-2 infection (Cafiero et al., 2021; Çelik et al., 2021; Möhlendick et al., 2021). Additionally, the *ACE* gene has a 287 base pair (bp) insertion/deletion (I/D) polymorphism in intron 16 which has been reported to influence changes in the levels of ACE in the blood and tissues (Delanghe et al., 2020). Individuals with the D/D genotype are said to have significantly higher levels of ACE and a reduced expression of ACE2 as compared to those with the I/D or I/I genotypes (Rigat et al., 1990). The high expression of ACE can influence COVID-19 severity risk due to decreasing ACE2 receptor levels (Çelik et al., 2021; Verma et al., 2021).

The genetic diversity of polymorphisms in the *ACE* and *ACE2* genes need to be investigated in the African population which will give insight into susceptibility for both communicable (malaria and COVID-19) and non-communicable diseases affecting the population. This study therefore reports the genetic diversity of *ACE* and *ACE2* gene polymorphisms in the Ghanaian populace and the implications for susceptibility to or protection from diseases of public health importance.

2. Materials and methods

2.1. Study sites and samples

The study was conducted with archived samples from uncomplicated malaria patients from three regions in Ghana, namely Cape Coast (5.1315°N, 1.2795°W), Navrongo (10.8940°N, 1.0921°W) and Hohoe (7.1519°N, 0.4738°E). Three hundred archived filter blood blots collected from children aged ≤9years with uncomplicated malaria presenting at designated health centres were used for the study. These three regions are inhabited by different ethnic/tribal groups (Fantis in Cape-Coast, Ewes in Hohoe and Kassena in Navrongo; 100 samples in each group). The samples have been stored in plastic bags containing silica gels and kept at room temperature until use.

2.2. Ethics statement

Written informed consent was obtained from all parents and guardians of study participants approved by the Noguchi Memorial Institute for Medical Research Institutional Review Board (IRB CPN 032/05-06a amed. 2021). The consent also covered the future use of the archived samples for molecular analysis.

2.3. Laboratory molecular analysis (PCR and sanger sequencing)

DNA was extracted from dried blood blots using STR GO! Lysis buffer (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Cut a 3 mm diameter hole from the dried blood blot, add 100 µl of STR GO! lysis buffer and vortex for 10 s. Heat at 95 °C for 2 min and spin at full speed for 2 min. DNA is ready to use. To store, remove the filter paper and freeze at −20 °C. Use 2 µl per PCR reaction.

Conventional PCR was also performed to amplify the coding regions of the *ACE2* gene using a published protocol by Gómez et al. (2020) with minimal modification. PCR to detect the I/D polymorphism of the *ACE1* gene, the *ACE* rs2106809 (C/T) and rs2285666 (G/A) polymorphisms of the *ACE2* gene were performed using previously published protocols (Çelik et al., 2021). A 25 µl reaction mix contained 1 × PCR buffer, 3.0 mM $MgCl_3$, 0.25 mM dNTP mix (Biotools S.A., Spain), 0.3 µM primers (Sigma-Merck, Spain) and 1.5 U of Taq polymerase (Biotools S.A., Spain). Successfully amplified samples were Sanger sequenced in the forward and reverse directions. Primer sequences, PCR amplicon sizes and cycling conditions used are shown in Supplementary Table 1.

2.4. Data analysis

Quality sequences obtained were run in the Basic Local Alignment Tool (http://blast.ncbi.nlm.nih.gov/) to check for authenticity of the sequence data. Sequences were then analysed using CLC Genomic Workbench version 22.0.1 (Qiagen, Aarhus, Denmark) and Benchling.com (San Francisco, CA, USA). Sequences were aligned to the wild type reference ENSG00000130234 to identify the variants. Frequencies of mutations were determined for each sample by individual counts. Haplotype frequency (%) was calculated as:

$$\frac{\textit{Total no.of individuals with combination of both rs2106809(C/T) and rs2285666(A/G) alleles per site}}{\textit{Total number of individuals sampled per site}\, x\, 100}$$

Chi-square test (was used to test for significant difference in the frequencies of the alleles among the ethnic groups. Hardy-Weinberg equilibrium (HWE) was assessed for genotype deviations using the $\chi 2$ test. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. ACE1 polymorphisms in Ghanaians

The *ACE1* (I/D) polymorphism was analysed in a total of 300 samples (100 from each ethnic group. The DD genotype was the highest observed in all individuals at a frequency of 54% (162/300). Overall frequencies of the II and ID genotypes observed was 17.3% (52/300) and 28.7 (86/100) respectively. The DD genotype was mostly seen in the Fantis at a frequency of 62% (62/100) whilst the II genotype was the most prevalent in the Ewes at 18% (18/100). The distribution of the observed alleles in the groups and according to sex is shown in Table 1.

3.2. ACE2 rs2285666 (G/A) and rs2106809 (C/T) alleles in Ghanaians

A total of 300 samples, 100 each from each site were genotyped to

Table 1
Distribution of genotypes/alleles and haplotypes of ACE2 rs2285666, ACE2 rs2106809 and ACE I/D in the three ethnic groups.

Polymorphism	Gender	Allele/Genotype	Groups			χ^2	p-value
			Ewe %	Kassena %	Fanti %		
			n = 100	*n* = 100	n = 100		
ACE2	Male	*A*	13	13	18	0.439	0.8029
rs2285666		*G*	39	38	42		
	Female	AA	1	1	2	5.602	0.2309
		GG	33	33	19		
		AG	14	14	19		
		WT	0	1	0		
ACE2	Male	C	2	9	10	6.218	0.0446*
rs2106809		T	38	26	44		
	Female	CC	0	5	2	6.56	0.1610
		TT	24	22	24		
		CT	15	18	10		
		WT	21	20	10		
ACE2	All	G-C	1	11	1	31.787	<0.0001*
Haplotype		G-T	51	40	48		
		A-C	0	1	8		
		A-T	10	4	9		
ACE I/D	All	DD	44	56	62	8.335	0.0800
		II	18	17	17		
		ID	38	27	21		

WT: wildtype allele; – not assessed; * significant.
NB: *ACE* 2 rs2285666 and rs2106809 data for gender were analysed separately as male and female due to the reason that it is located on the X- chromosome with males being only hemizygous and females being homozygous or heterozygous.
All genotypes analysed for *ACE* I/D, *ACE2* rs228566 and rs2106809 wwere not in HWE.

determine these polymorphisms in the introns of *ACE2,* rs2285666 and rs2106809. Of the 300 samples genotyped for the rs2285666 polymorphism, the G allele was the predominant in 68% (68/300) of individuals followed by the A allele and AG genotype at 16.3% (49/300) and 15.3% (46/300) respectively. One individual (0.3%,1/300) had none of the alleles and thus was a wild type (WT). For the rs2106809 polymorphism, the T allele was observed to be the most prevalent allele in all individuals sampled at a frequency of 59.3% (178/300) whilst the C allele and CT genotype were observed in 9.3% (28/300) and 14.3% (43/300) respectively. The wildtype allele was seen in 17% (51/300) of individuals.

Analysis done using ethnicity due to the sites where samples were collected showed that for the rs2285666 polymorphism, the G allele was the most dominant observed across all the three ethnic groups. The Ewes had the highest frequency at 72% (72/100). For the rs2106809 polymorphism, the T allele was the highest observed across all three groups with the Fantis having the highest frequency at 68% (68/100). Table 1 shows the distribution of the observed rs2285666 and rs2106809 polymorphisms as seen in three ethnic groups. There was no statistical significance on the frequencies of the rs2228566 alleles across the three groups ($p = 0.4498$).

The haplotypes of alleles in the individuals for the two introns were also assessed. A total of 184 (61.1%) individuals had a combination of both rs2106809 and rs2285666 alleles. The G-T haplotype was seen in 46.3% (139/300), G-C in 4.3% (13/300), A-C in 3% (9/300) and A-T in 7.7% (23/300) of the individuals. The Ewes had the highest frequency of G-T at 51% (51/100). The distribution of all observed haplotypes is shown in Table 1.

3.3. Polymorphisms in ACE2 coding regions in Ghanaians

A total of 300 samples were sequenced after PCR amplification for exons 1–4. Genetic data analysis was conducted with 268, 282 and 137 good quality sequences for exons 1, 2 and 3–4 respectively. Most of the observed mutations occurred once and were unique to Ghanaians and were seen in exons 1 and 3–4. No variants were observed in exon 2 of *ACE2* of Ghanaians. The variant N121K (2.3%; 7/300), was the highest observed followed by D38N (2.0%; 6/300) and N121T (1.3%; 4/300). The distribution of the observed variants is shown in Table 2. The reported *ACE2* mutations linked to susceptibility to SARs-CoV-2 infections

Table 2
List of observed mutations in *ACE2* gene of Ghanaians.

Exon 1		Exons 3–4	
Mutation	No. of individuals	Mutation	No. of individuals
S5S	1	T118T	1
L9S	1	N121T	4
S10N	1	N121K	7
T15I	1	T122T	1
A16G	1	S128N	1
Q18Q	2		
Q18R	1		
S19P	1		
I21H	1		
I21L	1		
E22*	1		
E23G	1		
Q24T	1		
A25A	1		
A25G	1		
K26Q	1		
K26E	1		
K26I	1		
T27I	1		
T27T	1		
E35Q	2		
E37K	1		
D38N	6		
Y41H	1		
Q42H	1		
S47S	1		
T52P	1		
N53T	1		
E57Q	2		

*-stop codon; **bold**: known mutation.

Table 3
Previously reported *ACE2* mutations linked to susceptibility to SARs-CoV-2 infections and those observed in Ghanaians.

Study reference	Ethnicity	Reported polymorphisms	Relevance in disease progression	Observed polymorphisms at the same codon in Ghanaians (n)
Hattori et al., 2022; Suryamohan et al., 2021; Vadgama et al., 2022	Asians, Europeans	S19P	Increases host susceptibility to SARS-CoV-2 infection	**S19P** (1)
Hattori et al., 2022; Chen et al., 2021; Suryamohan et al., 2021	Americans, Asians, Europeans	I21V/T	Increases host susceptibility to SARS-CoV-2 infection	I21H (1), I21L (1)
Hattori et al., 2022	Asians, Europeans	E23K	Increases host susceptibility to SARS-CoV-2 infection	E23G (1)
Hattori et al., 2022	Asians, Europeans	A25T	Increases host susceptibility to SARS-CoV-2 infection	A25G (1); A25A (1)
Hattori et al., 2022; Vadgama et al., 2022; Chen et al., 2021; Suryamohan et al., 2021	Africans, Americans, Asians, Europeans	K26R	Increases host susceptibility to SARS-CoV-2 infection	K26Q (1), K26E (1), K26I (1)
Hattori et al., 2022; Chen et al., 2021	Asians, Europeans	T27A	Increases host susceptibility to SARS-CoV-2 infection	T27I (1), T27T (1)
Hattori et al., 2022; Vadgama et al., 2022; Suryamohan et al., 2021	Europeans	E35D/K	Decreases host susceptibility to SARS-CoV-2 infection	E35Q (2)
Hattori et al., 2022; Suryamohan et al., 2021; Bhattacharjee et al., 2021	Asians, Europeans, Africans, African Americans	E37K	Decreases host susceptibility to SARS-CoV-2 infection	**E37K** (1)
Suryamohan et al., 2021	Asians, Europeans	D38V	Decreases host susceptibility to SARS-CoV-2 infection	D38N (6)

n- number of samples with mutation; **bold**: same as previously reported mutation.

from other publications and those observed in Ghanaians are shown in Table 3.

4. Discussion and conclusion

Genetic factors play a vital role in determining an individual's susceptibility or resistance to disease. These factors also regulate disease progression and outcome (Andreakos et al., 2022). The *ACE* and *ACE2* receptor genes are reported to have multiple genetic variations that influence *ACE* enzyme expression and the consequent variation in disease severity (Çelik et al., 2021). This study thus reports polymorphisms in *ACE1* (I/D), *ACE2* rs2106809 and rs2285666 and *ACE2* exons 1–4 in Ghanaians. Our findings reveal the presence of polymorphisms associated with both communicable and non-communicable diseases and other novel mutations in both genes with no identified roles in disease susceptibility or severity.

The D allele of the *ACE1* gene has been associated with increased *ACE* levels in humans (Woods et al., 2000), and the allele was observed in 54% (162/300) of individuals studied. This corroborates a study by Sarangarajan et al. (2021), where it was seen to be in high frequencies in different populations, particularly in Africans, Europeans and Asians. This allele has also been linked to low cerebral malaria incidence in a study conducted in India (Dhangadamajhi et al., 2010). The high occurrence of this allele in the population studied could be as a result of being naturally selected overtime in the population due to the protective effect it provides against malaria. The increase in Ang II production resulting from this same allele has also been significantly associated with mild malaria (De et al., 2021a; Dhangadamajhi et al., 2010). The I allele of the *ACE* (I/D) polymorphism on the other hand has been linked to low levels of ACE in the blood. It inhibits RNA polymerase II which regulates mRNA expression or alternativelyby splicing which leads to a truncated ACE protein that lacks one of its active sites (Rigat et al., 1990; Mafra et al., 2018; Purwaningroom et al., 2015).

The observed high frequency (59.3%) of the *ACE2* rs2106809 T allele observed in the study is in line with previous studies conducted in India, (De et al., 2022; Gallego-Delgado et al., 2016; Dhangadamajhi et al., 2010) where this allele has been suggested to undergo significant positive selection. The T allele is in high proportions (>80%) in African populations and therefore the hypothesized protective effect it provides against malaria. Individuals with this allele are said to have reduced *ACE2* activity which results in increased Ang II and subsequently protecting against severe malaria by inhibiting *Plasmodium* growth and erythrocyte invasion (De et al., 2022; De et al., 2021b; Sampson et al., 2014; Saraiva et al., 2011; Dhangadamajhi et al., 2010). Therefore in a malaria-endemic country like Ghana, the observation of this allele in high frequencies is not surprising as people who live in such areas are said to be more likely to have a genetic variant of *ACE2* (De et al., 2021). When the individuals were groups according to ethnicity, a significant difference were observed for the distribution of the C/T alleles only and not for the other alleles of the *ACE*.

The combined effect of the D and T alleles with a resultant increased *ACE* expression will increase Ang II levels and increase antiplasmodial activity and protection against cerebral malaria (Dhangadamajhi et al., 2010; Maciel et al., 2008). It could therefore be suggestive that the high number of individuals possessing these alleles maybe due to a natural selection process with malaria as the selective pressure. The rs2285666 A allele was observed in a low frequency (16.3%) of the individuals sampled. It has been linked to increased ACE2 levels (Asselta et al., 2020), thus may protect against malaria pathogenesis.

The *ACE2* gene exons analyses revealed both known and novel mutations but at low frequencies. The observation of these mutations at low frequencies is not surprising as it corroborates a GnomAD population data report by Ellinghaus et al. (2020) where the *ACE2* gene was reported to be a gene with a low allele frequency of missense variants. The observed S19P and E37K mutations in the study is noteworthy as the study cohort comprised of malaria patients. This is because these mutations have been reported to influence binding affinity of the SARS-COV 2 virus (Hattori et al., 2022; Suryamohan et al., 2021). *Plasmodium* spp. are reported to invade RBCs using *ACE2* receptors located on the surface of the RBCs (Konozy et al., 2022). There is a possibility that the synergistic effect of both mutations could influence the susceptibility or resistance of both SARS-COV 2 and malaria co-infection. The observation of these mutations in the Ghanaian population may suggest that there is free movement of genetic material in the population including non-African population admixture (Kempińska-Podhorodecka et al., 2012). The highest occurring mutations, N121K (2.3%), D38N (2.0%) and N121T (1.3%) in the study population maybe indicative of the selection of these variants under malaria pressure and thus may influence susceptibility or resistance to the disease in the Ghanaian population. Interestingly, there were no mutations in the exon 2 of the *ACE2* gene in the Ghanaian populace.

Genetic variants in the *ACE* and *ACE2* genes observed in the Ghanaians were in line with geographical distributions worldwide, however with novel mutations were also observed. The observation of novel mutations may indicate that there is possible selection of these variants in the Ghanaian population. Further studies are warranted to determine the roles of the novel mutations with regard to SARsCOV2 and malaria infections.

Author contribution

NODQ and JMR conceptuatised the idea, NODQ and ML did the lab work, NODQ and POA did the sequence data analysis, NODQ drafted the manuscript, all authors reviewed the manuscript.

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CRediT authorship contribution statement

Nancy O. Duah-Quashie: Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Philip Opoku-Agyeman:** Writing – review & editing, Validation, Formal analysis, Data curation. **Marta Lanza:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Jose Miguel Rubio:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2024.105568.

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