Polymorphisms in Exon 13 of Angiotensin-Converting Enzyme Gene among Hypertensive Patients in Sudan

R. T. Osman, D. A. Hassan, M. I. Elamin, M. Elamin, D. Mursi, M. A. M. Salih

ABSTRACT

Background: The identification of the critical regions within angiotensin-converting enzyme (ACE) gene which predict hypertension and/or influence ACE activity would have significant implications for precision medicine. Studies investigating the association of ACE gene polymorphisms and the risk of developing hypertension have yielded inconsistent results.

Objective: The aim of the study is to identify single nucleotide polymorphisms (SNPs) or haplotype markers in exon 13 of ACE gene and their association with essential hypertension in a sample of Sudanese population.

Methods: Amplified fragments of 550bp across exon 13 of ACE gene were outsourced to the Macrogen Company, Seoul, South Korea for sequencing. Finch TV program was used to view the chromatogram. Gene sequences were translated into amino acid sequence, using GeneMark version 4.2. The structural effect of a point mutation in a protein sequence was analyzed using PROJECT HOPE online website. Linkage disequilibrium between polymorphic variants was determined using Haplovie v4.2.

Results: Seven polymorphisms of ACE gene were identified in the sequenced fragments: four exonic SNPs (Rs4316, rs4317, rs4318 and one unreported SNP); an intronic SNP (rs12720723); one SNP at the intronic-exonic boundary site (rs4320); and an intronic I/D (rs4319). Haplotype analysis identified two blocks within 550bp spanning area of the ACE gene. Both blocks were composed of six SNPs: rs12720723; unreported SNP; rs4316; rs4317; rs4318 and rs4319. Each block consisted of five haplotype structures. Block 1 included B1-H1 (GCC), B1-H2 (ACC), B1-H3 (GCT), B1-H4 (GAT) and B1-H5 (AAT), whereas block 2 included B2-H1 (TAC), B2-H2 (CGC), B2-H3 (TAA), B2-H4 (CAC) and B2-H5 (TGC). Rs4317 and rs4318 were in moderate to high LD and displayed relatively high MAF among hypertensive participants. Rs4316; rs4319 and rs4320 were in moderate to high LD and displayed relatively high MAF among hypertensive participants.

Conclusion: The results of our study suggest that the 3 SNPs within exon 13 of the ACE gene (rs4316, rs4319 and rs4320) could be genetic markers for developing hypertension as evidenced by the high LD and MAF observed in hypertensive participants. Moreover, rs4318 being in LD with rs4317 could highlight the importance of block 2 in predicting hypertension among blacks.

Keywords: ACE gene, essential hypertension, haplotype view, linkage disequilibrium, polymorphisms.

I. INTRODUCTION

Hypertension is a well-established, independent risk factor for cardiovascular diseases, stroke, and end-stage renal disease [1]. Hypertension is more severe, resistant to treatment and likely to lead to immediate end organ damage and premature death in African patient population [2], [3]. According to a survey conducted by the Sudanese society of hypertension in 2018, the prevalence of essential hypertension among the adult Sudanese population is 28.2% [4].

The renin angiotensin aldosterone system (RAAS) plays a critical role in maintaining fluid and salt balance and regulation of blood pressure [5]. The identification of the critical regions within angiotensin-converting enzyme (ACE) gene which predict hypertension and/or influence ACE activity would have significant implications for precision medicine. Studies investigating the association of ACE gene polymorphisms and the risk of developing hypertension have yielded inconsistent results. The functional effect of ACE I/D polymorphism is not known but the polymorphism is thought to be an “anonymous” marker in linkage disequilibrium with a functional variant which directly influences ACE levels [6]-[8]. However, ACE I/D polymorphism is reported to be associated with essential hypertension among patients from
Sub-Saharan Africa [9]. Reference [10] proposed that other candidate polymorphisms within this gene may contribute to the mechanism affecting plasma ACE activity and development of hypertension. Reference [11] reported that polymorphisms in the ACE gene influence the activity of ACE as well as the blood pressure response to ACE inhibitors. As revealed by measured haplotype analysis, [12] reported two major quantitative trait loci associated with ACE activity between exon13 and intron18 and between intron 20 and 3’ UTR.

This study aimed to identify single nucleotide polymorphisms (SNPs) or haplotype markers in exon 13 of ACE gene and their association with essential hypertension in a sample of Sudanese population.

II. METHODS

Participants were recruited from Friendship Hospital (Khartoum, Sudan). Hypertension was defined as a systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg, or the subject is currently receiving antihypertensive treatments. Patients with secondary hypertension, renal, liver or cardiac abnormalities were excluded from the study.

Amplified fragment of 550bp across exon 13 of ACE gene were outsourced to the Macrogen Company, Seoul, South Korea for sequencing. Finch TV program, (http://www.geospiza.com/Products/finchtv.shtml) was used to view the chromatogram. Sequence identities to human genome was confirmed using nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi.). Polymorphic SNPs selected via aligning the sequence of hypertensive participants (0.24, 0.30 and 0.42 respectively, Table I). This deviation may be attributable to high heterogeneity in hypertensive patients.

Haplotype analysis showed two blocks within 550bp spanning area of the ACE gene. Both blocks were composed of six SNPs: rs12720723; unreported SNP; rs4316; rs4317; rs4318 and rs4319. Interestingly, homozygous mutant alleles of all polymorphisms were only observed among hypertensive patients.

Each block consisted of five haplotype structures. Block 1 included B1-H1 (GCC), B1-H2 (ACC), B1-H3 (GCT), B1-H4 (GAT) and B1-H5 (AAT), whereas block 2 included B2-H1 (TAC), B2-H2 (CGC), B2-H3 (TAA), B2-H4 (CAC) and B2-H5 (TGC). B1H2 (A-C-C) was the second most common haplotype where, the frequency of the A allele of (rs12720723) was higher than the G allele. (T-A-I) was the second common haplotype in B2H3. Insertion allele of (rs4319) was higher than deletion allele. However, B2H2 haplotype (C-G-D) carried two less common alleles; C allele of (rs4317) and G allele of (rs4318) (see Fig. 1).

Rs4317 and rs4318 were in moderate linkage disequilibrium (LD) (D’ value=0.69) among hypertensive patients. As shown in Fig. 2, three SNPS (rs4316; rs4319 and rs4320) were in moderate to high LD and displayed relatively high MAF among hypertensive participants (0.24, 0.30 and 0.42 respectively, Table I).

### Table 1: Genotype Frequencies of ACE Polymorphisms

| SNP ID     | Alleles | MAF | ObsHET | Pred HET | HW p value |
|------------|---------|-----|--------|----------|------------|
| rs12720723 | G/A     | 0.231 | 0.308  | 0.355    | 1.0        |
| Unreported SNP | C/T | 0.042 | 0.083  | 0.08     | 1.0        |
| rs4316     | C/T     | 0.24  | 0.16   | 0.365    | 0.0206     |
| rs 4317    | T/C     | 0.121 | 0.182  | 0.213    | 0.7623     |
| rs 4318    | A/G     | 0.147 | 0.294  | 0.251    | 0.9236     |
| rs 4319    | T/D     | 0.303 | 0.364  | 0.422    | 0.6189     |
| rs4320     | G/A     | 0.426 | 0.029  | 0.489    | 0.000      |

Fig. 1. Haplotype frequencies between six SNPs in the ACE gene. In the crossing areas, a value of multiallelic D’ is shown to represent the level of recombination between the two blocks.

Fig. 2. Linkage disequilibrium of the SNP markers in the ACE gene. Standard color scheme is used to display LD with bright red color for very strong LD (LOD = 2 D’ = 1), white color for no LD (LOD<2, D'=1), pink red (LOD = 2 D'<1), and blue (LOD<2 D' = 1) for intermediate LD.
IV. DISCUSSION

This study identified seven SNPs polymorphisms and two blocks of five haplotype structures within exon 13 of the ACE gene. The diverse haplotype structure and short blocks observed in the study population is consistent with a previous study which reported shorter haplotype blocks and greater haplotype diversity among Africans compared to Europeans [13]. In contrast, a measured haplotype-based case-control study of 31 ACE polymorphisms among Chinese hypertensive patients reported no observed LD between rs4316 and rs4320 [12]. However, the study did show that rs4316 was in LD with other genetic variants. According to NCBI database, rs4316 is a synonymous variant in NP_0011711528.1 (Pro27). Hence, rs4316 would not directly alter ACE protein structure or function. However, being in LD with rs4319 and rs4320 among hypertensive patients suggests that rs4316 may play a role in predisposing to hypertension. This suggestion is further supported by the presence of high frequency (0.15) of TT homozygous variant genotype among hypertensives compared to none among controls. Rs4316 has also been reported to have a missense effect in NP_690m043.1 and NP_001171528.1 isoforms which may affect the activity of plasma ACE. Of note, in XP_006721800.2 ACE isoform, rs4316 has a stop gain effect which disrupts the translation process of ACE. An intrinsic insertion/deletion polymorphism of rs4319 may influence the regulation process of ACE gene. Being in LD with upstream rs4316 and rs4320 and having high haplotype frequency among hypertensives highlights its potential role in developing the disease. However, this SNP is not reported in ClinVar and limited data is available on its association with hypertension. Rs4317 and rs4318 were in moderate LD (D* value=0.69) among hypertensive patients. Rs4317 alters the serine 32 into proline or isoleucine 84 into threonine in NP_001171528.1 and XP_006721800.2 isoforms respectively (http://www.ncbi.nlm.nih.gov/SNP). The alteration of serine 32 into proline introduces a more hydrophobic residue at this position and therefore leads to loss of hydrogen bonds and/or disturbance of the correct folding of the ACE protein. In XP_006721800.2 isoform, threonine is smaller in size than the original isoleucine residue located at the surface of a domain of unknown function. This may cause possible loss of external interactions and loss of hydrophobic interactions with other molecules on the surface of the protein (project hope online website (www.cmbi.ru.nl/hope)).

On the other hand, Rs4318 can be considered as a missense mutation in NP_001171528.1 isoform. Such a mutation could substitute serine to glycine at position 49. It is known that glycine is very flexible and can disturb the required rigidity of the protein at this position (http://www.cmbi.ru.nl/hope). Notably, rs4318 constituted a block with other ACE polymorphisms in African American patients and was absent among White subjects [13]. In contrast to our result, rs4318 was not associated with hypertension in a haplotype-based case-control study conducted in the Mexican population [14]. Hence, the association of rs4318 with hypertension might be limited to the black population. The presence of rs4318 in our study (conducted in a black African population) and being in LD with rs4317 could highlight the importance of block 2 in predicting hypertension.

Limitation of the study includes the small sample size. A future large scale study, which includes all ethnic groups representing Sudanese population, is recommended.

In conclusion, the results of our study suggest that the 3 SNPs within exon 13 of the ACE gene (rs4316, rs4319 and rs4320) could be genetic markers for developing hypertension as evidenced by the high LD and MAF observed in hypertensive participants. Moreover, rs4318 being in LD with rs4317 could highlight the importance of block 2 in predicting hypertension among blacks. While these results are intriguing, larger studies are needed to confirm and expand on these findings.

CONFLICT OF INTEREST
Authors declare that they do not have any conflict of interest.

REFERENCES

[1] Lifton RP. Molecular genetics of human blood pressure variation. Science. 1996; 272(5262): 676-80.
[2] Adgun AQ, Ishola DA, Akintomide AO, Ajayi AA. Shifting trends in the pharmacologic treatment of hypertension in a Nigeria tertiary hospital: a real-world evaluation of the efficacy, safety, rationality, and pharmacoeconomics of old and newer antihypertensive drugs. J Hum Hypertens. 2003; 17: 277-285.
[3] Optie LH, Seedat YK. Hypertension in sub-Saharan African populations. Circulation. 2005; 112: 3562-3568.
[4] Beheiry HM, Abdalla AA, Fahal NA, Mohamed MI, Ibrahim DA, Medani SA, et al. May Measurement Month 2018: an analysis of blood pressure screening results from Sudan, European Heart Journal Supplements. 2020, 22: H122–H124.
[5] Maluf-Meiken LC, Fernandes FB, Aragão DS, Ronchi FA, Andrade MC, Franco MC, et al. N-domain isoform of Angiotensin I converting enzyme as a marker of hypertension: population study. Int J Hypertens. 2012; 2012: 581780.
[6] Ji LD, Zhang LN, Shen P, Wang P, Zhang YM, Xing W, et al. Association of angiotensinogen gene M235T and angiotensin-converting enzyme gene I/D polymorphisms with essential hypertension in Han Chinese population: a metaanalysis. J Hypertens. 2010; 28: 419–428.
[7] Zarouk WA, Hussein JR, Esmacil NN, Raslan HM, Reheim HAA, Moguib O, et al. Association of angiotensin converting enzyme gene (E/D) polymorphism with hypertension and type 2 diabetes. Bratisl Lek Listy. 2012; 113(1): 14–18.
[8] He Q, Fan C, Yu M, Wallar G, Zhang ZF, Wang L, et al. Associations of ACE gene insertion/deletion polymorphism, ACE activity, and ACE mRNA expression with hypertension in a Chinese population. PLoS One. 2016; 11(5): e0156564.
[9] Mengesha HG, Petrucka P, Spence C, Tafesse TB. Effects of angiotensin converting enzyme gene polymorphism on hypertension in Africa: A meta-analysis and systematic review. PLoS one; 2019; 14(2): e021054.
[10] Zhu X, McKenzie CA, Forrester T, Nickerson DA, Broeckel U, Schunkert H, et al. Localization of a small genomic region associated with elevated urinary free metanephrines. American journal of human genetics.2000; 67(5): 1144–1153.
[11] Lifjeldahl U, Karlsson J, Melhus H, Kurland L, Lindersson M, Kahan T, et al. A microarray minisequencing system for pharmacogenetic profiling of antihypertensive drug response. Pharmacogenomics. 2003; 13(1): 7-17.
[12] Chung CM, Wang YF, Fann CS, Chen JW, Jong YS, Jou YS, et al. Fine-mapping angiotensin-converting enzyme gene: separate QTIs identified for hypertension and for ACE activity. PLoS One. 2013; 8(3): e56119.
[13] Zhu X, Chang YP, Yan D, Weder A, Cooper R, Luke A, et al. Association between hypertension and genes in the rennin-angiotensin system. Hypertension. 2003; 41: 1027–1034.
[14] Marti-niez-Rodr}guez N, Pouadas-Romo C, Villarreal-Melina T, Vallejo M, Del-Valle-Mondrago{n} L, et al. Single Nucleotide Polymorphisms of the Angiotensin-Converting Enzyme (ACE) Gene Are Associated with Essential Hypertension and Increased ACE Enzyme Levels in Mexican Individuals. PLoS ONE. 2013; 8(5): e65700.