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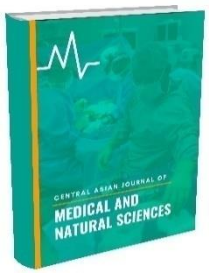

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Association of Carbamoyl Phosphate Synthase-1 Polymorphism (RS1047891) with Primary Hypertension among Hypertensives in Ibadan South-West Nigeria

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Abstract: Carbamoyl phosphate synthase 1 (CPS1) is a key gene in the first step of urea cycle. A functional single nucleotide polymorphism C/A has been reported to be associated with high levels of homocysteine. Increase in the homocysteine levels result to increase in hypertension most especially among the aged. This study, thus investigated the association of CPS1 (rs1047891) with primary hypertension with reference to a global simulated data.

A total of 100 participants, 50 normotensives were matched with 50 hypertensives within 18-80 years of age in Ibadan, South-west Nigeria were studied. A total of 2000 simulated global data set was used having 382 as normotensives and 1618 as hypertensives. Blood samples of participants from Ibadan were taken and other clinical parameters such as age, sex weight, height, systolic blood pressure and diastolic blood pressure were determined. The participants were genotyped for one SNP variant (rs1047891) using polymerase chain reaction and enzymatic restriction. Statistical analysis was performed using Haploview 4.2, Plink and SPSS ® 20.0 software for windows and p-value set at $p<0.05$.

The minor allele frequency of rs1047891 of CPS1 gene was similar to that in the hapmap database among the Yoruba population. The distribution of rs1047891 of CPS1 gene and n4_3, n4_4, m4_0 and qr2_4 of the simulated global data set genotypes was consistent with the Hardy-Weinberg equilibrium ($P>0.05$). There was no statistically significant association ($p>0.05$) between the polymorphism of CPS1 gene and SNPs in the simulated global data set with hypertension.

Key words: Nigeria, Polymorphism.

However, there was a statistically significant risk association between rs1047891 and hypertension when age and SBP are used as covariates, suggesting them as factors that influence the predisposition to hypertension. DBP, BMI and sex for rs1047891 showed no statistical significance but was significant for SNPs (n4_3, n4_4, m4_0 and qr2_4) in the simulated global data set.

These findings of this study substantiate the role of CPS1 (rs1047891) in the outcome of hypertension. Further large- scale studies across multiple populations will be required to reassess the association

Background

About one billion people are hypertensive worldwide and this number is projected to reach 1.54 billion by 2025 (Kearney *et al.,* 2005). Hypertension has been associated with heightened risk of cardiovascular morbidity and mortality. It is estimated that about 12.8% (9.4 million) of the global mortality figures can be attributed to hypertension and its complications (WHO, 2013).Primary hypertension is four times more widespread in black than white people (Lindhorst *et al.,* 2007; Loscalzo *et al.,* 2008).Numerous studies have shown that hypertension in sub-Saharan Africa (SSA) is a widespread problem, with a reported prevalence as high as 38 % (Opie and Seedat, 2005; Addo*et al.,* 2007; Steyn *et al.,* 2008; Ataklte *et al.,* 2015).

In adolescents, hypertension is still a growing public health problem due to the difficulty in detecting blood pressure unlike in adults (Oluremi *et al.,* 2017). Joseph and Bonita (2011), estimated prevalence among them to be 3.5% compared to Oluremi *et al.,* 2017 who gave a prevalence of 6.1% due to increase in body weight. Only about half of the population with hypertension is aware of their hypertension, showing a high burden of undiagnosed and untreated hypertension in SSA (David *et al.,* 2015).In Nigeria, the prevalence of hypertension is high due to the large populace of the country showing an overall prevalence of 33.1% in Ibadan (Adeloye *et al.*,2015; Ikeoluwapo *et al.,* 2016).

Hypertension a silent killer (William, 2018),is the most common non-communicable disease in the world (Gidding, 2006). It is characterized with persistent increase in systolic and diastolic blood pressure (WHO, 2019). It has no identifiable origin, but results from a disorder of systems regulating blood pressure (BP) such as several local and circulating neurohumoral and vasoactive factors (Hayet, 2012). It is a multifactorial and polygenic disease resulting from the interaction between genetic and environmental factors (Lifton *et al.,* 2001).It is also a risk factor for stroke and a vital public health problem (Zbigniew *et al.,* 2013). It cuts through every social class, having both lower and higher-income groups at increased risk (Opie and Seedat, 2005). The risk factors for hypertension includes but not limited to age, diet, body mass index (BMI), positive family history, tobacco use and genetic factors (Tamaki *et al.,* 2002; Dzau *et al.,* 2006 and Yi *et al.,* 2006).

The major form of hypertension in a population occurs with no clear cause and therefore is categorized as primary hypertension while others which occur usually as a complication of other conditions are identified as secondary hypertension. Secondary hypertensive cases accounts for about 5% of total hypertension prevalence categorized as secondary hypertension (Carretero and Oparil, 2000). The indications of hypertension are mostly minor or the patient remains asymptomatic until the disease develops (Williams, 2007). It rises with age and aggregates with other cardiovascular risk factors such as obesity, dyslipidemia, hyperinsulinaemia, among others (Madhu, 2012). Increase in systolic blood pressure with advancing age leads to hypertension whereas diastolic blood pressure tends to remain constant or decline with advancing age as a result of large artery stiffening (Germaine, 2014 and Ayinde et al., 2022). The use of simulated data can help verify with the actual data, factors that predispose to the development of hypertension, providing better decision making in early detection of hypertension and thereby reducing the associated death (Idowu, 2017).

Several genetic studies have been done globally in relation to hypertension, identifying several polymorphisms on different genes thought to be linked with hypertension (Kidambi *etal.*, 2012). The Genome Wide Association Studies (GWAS) done on African Americans discovered polymorphisms in candidate genes such as PMS1, SLC24A4, STK39 among others, although independent studies carried out to validate these claims have given inconsistent results (Kidambi *et al.*, 2012).Silene *et al.*, (2015) reported the presence of rs1047891 in CPS1 gene among 13974 healthy Argentine Caucasian women. The single nucleotide polymorphism rs1047891 has been associated with increased homocysteine level which has also been linked with cardiovascular diseases. Therefore, this study is set out to investigate the association between the SNP and hypertension among hypertensive patients in South-western Nigeria. A comparison with simulated global data will help validate findings in this study.

Hypertension is one of the leading causes of death worldwide. Over three quarters of cardiovascular disease deaths occur in low-and middle-income countries and future projections are not favorable. Nigeria with her highest population in the region is the worst hit by the condition. The increasing prevalence, high associated mortality and the consequential great socio-economic burden of hypertension has made it a disease of public health importance. It is not only a problem among the aged but also in younger adults and adolescents due to factors such as overweight, obesity, among others now in developing countries.

Several studies have shown many gene variants to have an effect on the development of hypertension and susceptibility to primary hypertension (Amballi A., Ayinde A., Asaolu O., & Olabumyi O., 2022). Reports have previously established an association between rs1047891 of CPS1 gene and predisposition to cardiovascular disease among the Caucasian population. However, there is no documented report on the association amongst the black population especially of African descent even though its minor allele frequency (MAF) of rs1047891 shows the presence of the SNP among the African population.

Hypertension is a major health concern globally especially in developing countries with a marked burden in sub-Saharan Africa. Projected figures on prevalence and mortality associated with hypertension in this region of the world has necessitated the need for drastic interventionto avert an impending public health disaster. Hypertension is still a growing public health problem among adolescents which if not managed early, will persist till adulthood. This study however focused on adults because of the high prevalence of hypertension in them since increase in age is a risk factor that predisposes to the development of hypertension.

Polymorphisms of CPS1 gene have shown to have effect majorly in the aged which is associated with increased homocysteine levels and consequentially higher predisposition to hypertension among several populations in the world. However, this association is yet to be established among Africans. Findings of this study on the association of rs1047891 and hypertension will form part of the basis for genetic screening among adolescents to provide preventive strategies that can help reduce hypertension in them, improved drug development to achieve better therapeutic outcomes among patients in this region of the world. This study will also help validate the generalization of previous Genome Wide Association Studies done on this SNP to the population in this region of the world.

Analysis of the simulated global data set was also carried out to help provide more insight to determine the likely effect of the SNP under study (rs1047891) with hypertension to the result seen in the simulated global data set.

Research question

Is there an association between rs1047891 of CPS1 gene and hypertension among hypertensive patients in Ibadan, South-west Nigeria?

Hypothesis

H_0: There is no association between rs1047891 of CPS1 gene among hypertensive patients in Ibadan, South-west Nigeria**.**

H_0: There is no association between the SNPs (n4_3, n4_4, m4_0 and qr2_4) of the simulated global data with hypertension.

General objective

To determine the association between rs1047891of CPS1 gene and hypertension among hypertensive patients in Ibadan, South-west Nigeria.

Specific objectives

The specific objectives of this study are to;

1. Determine the frequency of rs1047891 in Ibadan, South-west Nigeria;
2. Investigate the association of rs1047891with hypertension in Ibadan, South-west Nigeria;
3. Determine the frequency of n4_3, n4_4, m4_0 and qr2_4 in the simulated global data set;
4. Investigate the association between n4_3, n4_4, m4_0 and qr2_4 SNPs of the simulated data set and its risk of developing hypertension;
5. Determine the linkage disequilibrium between n4_3, n4_4 and m4_0 SNPs; and
6. Compare the association of hypertension with rs1047891 and n4_3, n4_4, m4_0 and qr2_4 SNPs of the simulated global data sets.

Material and Method

This study was carried out in Ibadan, South- west Nigeria. Ibadan is located in the south- eastern part of Oyo State at about 119km northeast Lagos and 120km east of Nigerian border with Republic of Benin and lies between latitude 7.401962^0N, latitude 3.917313^0E and at an altitude of 275 above sea level (Rafiu *et al.,* 2016).The study was an unmatched case control study design. The hypertensive patients are grouped as cases while the normotensives are grouped as controls. Comparison between the SNPs in the simulated global data comprised of unmatched cases and controls. This study was carried out at the University College Hospital (UCH), Ibadan North Local Government Area of Oyo state. The center is a tertiary health care provider that serves patients from all states in the South-western region of the country and beyond. It also houses adequate equipment that aid easy collection and analysis of data collected. Patients within the ages of 18 to 80 years were recruited at the University College Hospital, Ibadan as at the time of the study. A total of 50 hypertensives (cases) were recruited from the Medical Out-Patient Department (MOPD) and a total of 50 normotensives (control) from the General Out-Patient Department (GOPD) into the study using systematic random sampling method making a total of 100 patients.

3.6 Data collection techniques

3.6.1 Checking of blood pressure

Blood pressure was measured using mercury sphygmomanometer. The measurements were taken in the sitting position with exposed outstretched right arm on a table after resting for at least 5 min. Blood pressure was measured thrice for each person in the same visit with an interval of 5-10 minutes between measurements. The average of the last two measurements was then estimated as the blood pressure level of the subject. The participants were weighed using a bathroom weighing scale with light clothing and no shoes. The participants were made to stand erect and their heels in contact with

the wall. The height was determined using a stadiometer. A plastic tape was placed around the waist of participants and the measurement determined. A plastic tape was placed around the hips of participants and the measurement determined. Socio-demographic indices was obtained through the administration of questionnaires.

Statistical analysis

The anthropometric indices were analyzed using a Statistical Package for Social Sciences version 20.0 software for Windows (SPSS Inc., Chicago, USA). Multivariate analysis was performed using logistic regression, adjusting for age and gender, test for Hardy-Weinberg equilibrium (HWE) and frequency determination was done with Plink. Haploview 4.2 was used to determine the linkage disequilibrum. Statistical significance was calculated using a p value of 0.05.

Ethical consideration

This study was approved by the Oyo State Ethics Review Committee with AD Protocol no: AD 13/ 479/1082 (Appendix I). A signed written informed consent was obtained from the parents/ guardian of all participants as the rights according to the ethics code were explained and duly observed (Appendix II).

RESULTS

4.1 Descriptive and clinical parameters of participants from Ibadan Southwest Nigeria

A total of 100 participants comprising of 50 normotensives and 50 hypertensives were recruited for this study. 42 males and 58 females enrolled: 22 males and 28 females as controls and 20 males and 30 females as cases. However,50 (23 controls and 27 cases) participants were genotyped in the study.There is a statistically significant difference in the mean age for the study group with the normotensive and hypertensive group having 43.27 ± 2.274 and 57.61 ± 1.551 respectively.

Table 4.1 shows the descriptive and clinical parameters of the study participants in Ibadan, South-west Nigeria. The mean systolic pressure and diastolic blood pressure was significantly higher in the hypertensive group with a value of 146.92 ± 3.325 and89.02 ± 2.008($p<0.05$). The mean BMI was higher in the normotensive group and showed no statistically significant difference in the study group ($p>0.05$).

4.2 Genotypic and allelic frequency of CPS1 (rs1047891) polymorphism in Ibadan, South- west Nigeria

Table 4.2 shows the overall genotype and allele frequency of rs1047891 (CPS1). Homozygous Cgenotype had the highest genotype frequency of 0.70 while homozygous A genotype had the lowest genotype frequency (0.08). A minor allele frequency of 0.19 was observed for CPS1 (rs1047891) implying that allele A is the minor allele in the population. The reference minor allele frequency reported by NCBI is 0.283. The SNP is in Hardy-Weinberg equilibrium (pHWE > 0.05).

Table 4.1: Descriptive and clinical parameters of participants in Ibadan Southwest Nigeria

Parameter	Normotensive	Hypertensive	P-value
No of participants	50	50	
Gender			
Male Female	22 28	20 30	
Mean age (years)	43.27 ± 2.274	57.61 ± 1.551	<0.001*
Mean systolic blood pressure (SBP)(mmHg)	117.88 ± 2.184	146.92 ± 3.325	<0.001*

Mean diastolic blood pressure (DBP) (mmHg)	76.54 ± 1.516	89.02 ± 2.008	<0.001*
Mean weight (kg)	64.82 ±2.019	69.54 ±2.146	0.112
Mean height (cm)	159.89 ±2.344	161.85 ±1.270	0.340
Mean BMI(kg/m^2)	27.59 ± 2.780	26.57 ± 0.776	0.724

Significance set at p≤ 0.05, *statistical significance, BMI: Body mass index

Table 4.2: Genotypic and allelic frequency of CPS1 (rs1047891) polymorphism in Ibadan, South-west Nigeria

SNP	Genotype Frequency	Allele	Allelic Frequency	pHWE	MAF	Ref MAF
rs1047891						
AA	0.08	A	0.19	0.055*	0.19	0.283
AC	0.22					
CC	0.70	C	0.81			

*pHWE>0.05, HWE: Hardy-Weinberg equilibrium, MAF: Minor allelic frequency

4.3 Distribution of the genotypes of CPS1 (rs1047891) in association with hypertension in Ibadan, South-west Nigeria

Table 4.3 shows the distribution of CPS1 (rs1047891) genotype and the association with hypertension.CC and AA had the highest and lowest genotype frequency of 70.0% and 8.0% respectively in the population. AA was totally absent among the normotensive group although it had 14.8% frequency (n=4) in the hypertensive group. The test result did not show any statistical significance to confer a risk in the pairwise comparison between the normotensives and hypertensives(p=0.2005, OR=2).

4.4 Genetic analysis models and sex, age, BMI, SBP and DBP adjustment values for CPS1 (rs1047891)

The test analysis in table 4.4 shows the genetic analysis models and sex, age, BMI, SBP and DBP adjustment values for CPS1 (rs1047891). There was no statistical significant association between the genotype and the phenotype in both additive (p=0.274, OR=1.705) and dominant (p=0.718, OR=1.259) inheritance models in the multivariate analysis using sex, BMI, SBP and DBP as covariates. However, there was a statistically significant risk association between rs1047891 and hypertension when age is used as a covariate in both additive (p=0.00165, OR=1.098) and dominant (p=0.00136, OR=1.100) models but it was not significant for sex (p=0.212, OR=0.457; p=0.301, OR=0.534), BMI (p=0.988, OR=0.999; p=0.907, OR= 0.993), SBP (p=0.1773, OR=1.034; p=1.034, OR=0.907) and DBP (p=0.0926, OR=1.070; p=0.1003, OR=1.068) in both the additive and dominant models respectively.The test analysis showed no information on the recessive model.

Table 4.3: Distribution of the genotypes of CPS1 (rs1047891) with hypertension in Ibadan, South-west Nigeria

Genotype	Total genotype frequency	Normotensive	Hypertensive	P-value	OR
AA	4 (8.0%)	0	4 (14.8%)		
AC	11 (22.0%)	6 (26.1%)	5(18.5%)	0.2005	2
CC	35 (70.0%)	17 (73.9%)	18 (66.7%)		

Significance set at p≤ 0.05, OR:Odds ratio, CI: Confidence interval

Table 4.4: Genetic analysis models and sex, age, BMI, SBP and DBP adjustment values for CPS1 (rs1047891)

SNP	Minor Allele	Genetic Model	OR	p-value	SEX OR	SEX p-value	AGE OR	AGE p-value	BMI OR	BMI p-value	SBP OR	SBP p-value	DBP OR	DBP p-value
rs1047891														
	A	Add	1.705	0.274	0.457	0.212	1.098	0.00165*	0.999	0.988	1.034	0.1773	1.070	0.0926
	A	Dom	1.259	0.718	0.534	0.301	1.100	0.00136*	0.993	0.907	1.034	0.1784	1.068	0.1003
	A	Rec	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

OR: odds ratio, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, Significance set at p≤ 0.05

4.5 Linear regression analysis modelling the relationship between age and the dependent variables associated with hypertension for CPS1 (rs1047891)

The linear regression analysis modelling of table 4.5 shows the relationship between age and the dependent variables associated with hypertension for CPS1 (rs1047891). There was a linear relationship between age and SBP (p=3.69e-05); an increase in age results in an increase in SBPand also an inverse relationship with DBP (p=0.005286); an increase in age results ina decrease in DBP.There was no significant association between age and BMI in the additive, dominant and recessive models (p=0.5774, 0.7534, 0.4402) respectively.

4.6 Descriptive and clinical parameters of thesimulated global data

The study comprised of 2000 (841 males and 1159 females) participants. 382 of the participants were normotensives while 1618 were hypertensive. There was a statistically significant difference in the mean age for the study group with normotensives and hypertensive having 44.23±0.764 and 49.24±0.230 respectively.

Table 4.6 shows the descriptive and clinical parameters of the simulated global data. In the simulated global data, the mean systolic and diastolic was significantly higher in the hypertensive group (p<0.05). The mean BMI was higher in the hypertensive group although the difference did not show any statistical significance (p>0.05).

Table 4.5: Linear regression analysis modelling the relationship between age and the dependent variables associated with hypertension for CPS1 (rs1047891)

SNP	Independent Variable	Dependent Variable	Genetic model (beta) ADD	DOM	REC	Genetic model (p- value) ADD	D OM	REC
rs1047891	Age	BMI	0.2264	0.128	0.2975	0.5774	0.7534	0.4402
		SBP	0.5541	0.5587	0.5489	3.69e-05*	3.802e-05*	2.709e-05*
		DBP	-0.6037	-0.6078	-0.6024	0.005286*	0.00546*	0.004217*

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, Significance set at p≤ 0.05, *statistical significance

Table 4.6: Descriptive and clinical parameters of thesimulated global data

Parameter	Normotensive	Hypertensive	P-value
No of participants	382	1618	
Gender			
Male	382	459	
Female	0	1159	
Mean age (years)	44.23 ± 0.764	49.24 ± 0.230	<0.001*

Mean systolic blood pressure (SBP)(mmHg)	112.46 ± 0.669	157.54 ± 0.618	<0.001*
Mean diastolic blood pressure (DBP) (mmHg)	69.23 ± 0.436	99.61 ± 0.355	<0.001*
Mean weight (kg)	59.636±0.556	70.469±0.379	<0.001*
Mean height (cm)	1.647±0.004	1.609±0.002	062
Mean BMI(kg/m^2)	22.015 ± 0.203	27.28 ± 0.151	0.724

Significance set at p≤ 0.05 *statistical significance, BMI: Body mass index

4.7 Genotypic and allelic frequency of the simulated global data

All four SNPs in this study were found to be in Hardy- Weinberg equilibrium (pHWE ≥ 0.05). The genotypic, allelic and minor allelic frequencies of the SNPs are shown in Table 4.7.

4.8 Distribution of the genotypes in the simulated global data SNPs in the study population

The pairwise comparison between the normotensives and hypertensives showed no statistical significant association of the genotypic distribution for n4_3 (p=0.628), n4_4 (p=0.338), m4_0 (p=0.4682) and qr2_4 (p=0.2031). Table 4.8 shows the distribution of the genotypes in the simulated global data SNPs in the study population.

4.9 Genetic analysis models with sex, age, BMI, SBP and DBP adjustment values for the simulated global data SNPs

Table 4.9 shows the genetic analysis models with sex, age, BMI, SBP and DBP adjustment values for the simulated global data SNPs. There was no statistical significance in the additive, dominant and recessive models for n4_3 (p=0.629, OR=1.047; p=0.969, OR= 1.005; p=0.243, OR=1.369), n4_4 (p=0.347, OR=1.093; p=0.373, OR=1.109; p=0.569, OR=1.147), m4_0 (p=0.465, OR=0.941; p=0.687, OR=0.954; p=0.384, OR=0.869) and qr2_4 (p=0.204, OR=1.125; p=0.201, OR=1.158; p=0.542, OR=1.152) with hypertension.

However, there was a statistically significant risk association between n4_3 (p=0.00418, 1.031e-14, 1.720e-09 and 5.477e-24), n4_4 (p=0.00310, 1.733e-14, 2.734e-09 and 3.628e-24), m4_0 (p= 0.00322, 1.604e-14, 2.906e-09 and 2.246e-24) and qr2_4 (p=0.00304, 1.621e-14, 2.923e-09 and 2.867e-24) with hypertension in all genetic models using age, BMI, SBP and DBP as covariates respectively.

Table 4.7: Genotypic and allelic frequency of the simulated global data

SNP	Genotypic Frequency	Allele	Allelic Frequency	pHWE	MAF
n4_3					
TT	0.057	T	0.236	0.710	0.236
TA	0.358				
AA	0.586	A	0.764		
n4_4					
GG	0.064	G	0.239	0.110	0.239
GC	0.352				
CC	0.585	C	0.760		
m4_0					
AA	0.138	A	0.376	0.536	0.376
AT	0.386				
TT	0.476	T	0.624		
qr2_4					
GG	0.070	G	0.263	0.863	0.263
GA	0.386				
AA	0.544	A	0.737		

*pHWE> 0.05, HWE: Hardy-Weinberg equilibrium, MAF: Minor allelic frequency

Table 4.8: Distribution of the genotypes in the simulated global data SNPs in the study population

Genotype	Totalgenotype frequency	Normotensive	Hypertensive	P-value	OR
n4_3					
TT	114 (5.7%)	17 (4.5%)	97 (6.0%)		
TA	715 (35.75%)	141 (36.9%)	574 (35.5%)	0.628	1.047
AA	1171 (58.55%)	224 (58.6%)	947 (58.5%)		
n4_4					
GG	128 (6.4%)	22 (5.7%)	106 (6.6%)	0.338	1.096
GC	703 (35.1%)	129 (33.8%)	574 (35.5%)		
CC	1169 (58.5%)	231 (60.5%)	938 (57.9%)		
m4_0					
AA	276 (13.8%)	58 (15.2%)	218 (13.5%)	0.4682	0.9418
AT	772 (38.6%)	144 (37.7%)	628 (38.8%)		
TT	952 (47.6%)	180 (47.1%)	772 (47.7%)		
qr2_4					
GG	140 (7%)	24 (6.3%)	116 (7.2%)	0.2031	1.126
GA	772 (38.6%)	139 (36.4%)	633 (39.1%)		
AA	1088 (54.4%)	219 (57.3%)	869 (53.7%)		

Significance set at p≤ 0.05, OR: Odds ratio, CI: Confidence interval

Table 4.9: Genetic analysis models and sex, age, BMI, SBP and DBP adjustment values for the simulated global data SNPs

SNP	Minor Allele	Genetic Model	OR	p-value	AGE OR	AGE p-value	BMI OR	BMI p-value	SBP OR	SBP p-value	DBP OR	DBP p-value
n4_3	T	Add	1.047	0.629	1.206	0.00418*	1.199	1.031e-14*	1.056	1.720e-09*	1.173	5.477e-24*
	T	Dom	1.005	0.969	1.207	0.00375*	1.198	1.196e-14*	1.056	2.019e-09*	1.173	4.448e-24*
	T	Rec	1.369	0.243	1.206	0.00401*	1.197	1.215e-14*	1.056	2.135e-09*	1.174	3.595e-24*
n4_4	G	Add	1.093	0.347	1.027	0.00310*	1.196	1.733e-14*	1.055	2.734e-09*	1.174	3.628e-24*
	G	Dom	1.109	0.373	1.027	0.00276*	1.197	1.470e-14*	1.055	2.399e-09*	1.174	3.289e-24*
	G	Rec	1.147	0.569	1.027	0.00297*	1.196	1.499e-14*	1.055	2.935e-09*	1.175	1.977e-24*
m4_0	A	Add	0.941	0.465	1.027	0.00322*	1.196	1.604e-14*	1.055	2.906e-09*	1.175	2.246e-24*
	A	Dom	0.954	0.687	1.027	0.00311*	1.195	1.613e-14*	1.055	2.925e-09*	1.175	2.134e-24*
	A	Rec	0.869	0.384	1.027	0.00338*	1.196	1.662e-14*	1.055	3.072e-09*	1.175	2.474e-24*
qr2_4	G	Add	1.125	0.204	1.027	0.00304*	1.196	1.621e-14*	1.055	2.923e-09*	1.174	2.867e-24*
	G	Dom	1.158	0.201	1.027	0.00296*	1.196	1.517e-14*	1.055	2.911e-09*	1.174	2.740e-24*
	G	Rec	1.152	0.542	1.027	0.00312*	1.195	1.644e-14*	1.055	3.220e-09*	1.175	2.344e-24*

OR: odds ratio, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, *statistical significance, $p \leq 0.05$

4.10 Linear regression analysis modelling the relationship between age and the dependent variables associated with hypertension for the simulated global data

Table 4.10 shows the linear regression analysis between age and the dependent variables associated with hypertension for the simulated global data.There was a linear relationship between age and BMI (p=0.0003911, 0.0003969,0.0003848 and 0.0003757 respectively), SBP(p=2.033e-25,2.407e-25, 2.109e-25 and 2.283e-25 respectively)across the genetic models for n4_3, n4_4, m4_0 and qr2_4 and DBP under the additive model for n4_3 (p=0.1728) and dominant model for qr2_4 (p=0.1729); with increase in age resulting in a corresponding increase in BMI, SBP and DBP. An inverse relationship was also observed between age and DBP across the genetic models for both n4_3(p= 7.662e-12) and m4_0 (p=7.862e-12), the dominant and recessive model for n4_4(p=7.836e-12 and 8.492e-12) and the additive and recessive model for qr2_4 (p=8.042e-12 and 8.169e-12); as age increases, DBP decreases.

4.11 Linkage analysis

The n4_3, n4_4 and m4_0 SNPs are located on chromosome 6, having a LOD (measure of confidence) of 0.01, r^2 of 0.0 and D' (normalized linkage disequilibrium measure) of 0.011showing that a strong evidence of recombination exists between the SNPs and are therefore not in linkage disequilibrium in the study population.

4.10: Linear regression analysis modelling the relationship between age and the dependent variables associated with hypertension for the simulated global data

SNP	Independent Variable	Dependent Variable	Genetic model (beta) ADD	Genetic model (beta) DOM	Genetic model (beta) REC	Genetic model (p-value) ADD	Genetic model (p-value) DOM	Genetic model (p-value) REC
n4_3		BMI	0.14	0.1402	0.1405	0.0003911*	0.0003858*	0.0003731*
	Age	SBP	0.1634	0.1635	0.1631	2.033e-25*	1.968e-25*	2.488e-25*
		DBP	-0.173	-0.173	-0.1729	7.662e-12*	7.602e-12*	8.109e-12*
n4_4		BMI	0.13980.1397		0.1405	0.0003969*	0.0004026*	0.0003716*
	Age	SBP	0.1632	0.1633	0.1632	2.407e-25*	2.271e-25*	2.305e-25*
		DBP	0.1728	-0.1729	-0.1727	8.113e-12*	7.836e-12*	8.492e-12*
m4_0								
	Age	BMI	0.14020.1405		0.1411	0.0003848*	0.0003758*	0.0003516*

qr2_4	Age	SBP DBP BMI SBP DBP	0.1634 0.1634 0.1631 -0.1729 -0.1730 -0.1727 0.14040.1403 0.1406 0.1630.1633 0.1633 -0.1728 0.1729 -0.1728	2.109e-25* 2.008e-25* 2.415e-25* 7.862e-12* 7.704e-12* 8.47e-12* 0.0003757* 0.00003809* 0.000371* 2.283e-25* 2.15e-25* 2.317e-25* 8.042e-12* 7.842e-12* 8.169e-12 *

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, *statistical significance, p≤ 0.05

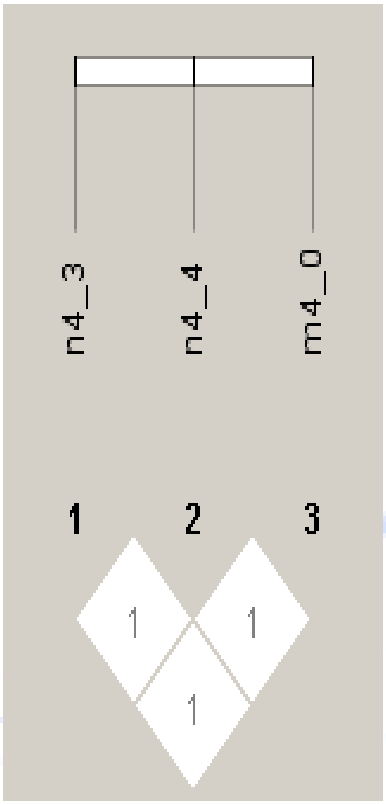

Fig 4.1: Linkage disequilibrium between n4_3, n4_4 and m4_0SNPs.

DISCUSSION AND CONCLUSION

Discussion

Hypertension is one of the leading causes ofmorbidity and mortality in the world (Donghao *et al.*, 2018). The aetiology of hypertension is complex due to the interaction of hereditary and environmental factors (Kunes and Zicha, 2009). It occurs when the mean arterial pressure is greater than the upper range of accepted normal pressure (Idowu, 2017). Hypertension has also been implicated in progressive glomerulonephritis, nephrosclerosis, among others (Mesrati, 2007). It is a highly inherited condition with its heritability value ranging from 15% to 35% (Kohara *et al.*, 2008).Hypertension cases are continuously on the increase in sub-Saharan Africa especially Nigeria due to exposure to risk factors of high blood pressure such as age, sex and lifestyle resulting to high morbidity and mortality rate (Idowu, 2017).

Development of hypertension is a characteristic feature of aging (Ostrakhovitch and Tabibzadeh, 2019) and has been positively associated with systolic and diastolic blood pressure (Lim and Cassano, 2002; Verdoia *et al.*, 2015).Aging results in decreased arterial and arteriolar elasticity due to atherosclerosis with consequent higher pumping pressure from the heart (Pinto, 2007). The descriptive characteristics of participants in Ibadan showed a statistically significant mean difference between

cases and controls with a higher mean age among hypertensives than in normotensives. Both systolic and diastolic blood pressure also showed to be significantly high among hypertensives having age, systolic and diastolic pressure as risk factors for development of hypertension. These findings are consistent with previous studies done by Zhang *et al.,* (2003) and Hang *et al.,* (2010).

Body mass index (BMI) is also an important factor to put into consideration when dealing with hypertension. Being overweight or obese are factors that predispose to hypertension. Overweight is body mass index between 25.0 to 29.9kg/m^2 and is said to increase blood pressure (Cornier *et al.,* 2011).A study by Wilbert (2017) comprising of Canadians aged 18 to 74 years showed that the prevalence of hypertension increased with increase in body mass index and vice versa.However, not all hypertensive patients are overweight and this is in line with studies done by Zhang *et al.,* (2013) and Shuo *et al.,* (2018).Participants in the Ibadan study group showed to be all overweight but this was high among normotensives showing no statistical significance.This is in contrast with Katia *et al.,* (2011) who showed both participants to be overweight but was high among hypertensives.

Genetic polymorphism in the CPS1 gene have been associated with increase in plasma homocysteine level which occurs due to the substitution of asparagine for threonine at the region critical for N-acetyl-L-glutamate binding (Summar *etal.,* 2004). Elevated plasma homocysteine (Hcy) concentration is considered a risk factor for cardiovascular disease and also associated with hypertension.Up-regulation of homocysteine also contributes to the development of age-associated disorders, loss of regenerative ability, cardiovascular dysfunctionand decline in renal and cognitive functions. The serum level of homocysteine increases with age and reaches 16.5+0.5 µmol/l in elderly people of 65 years of age with highest concentrations found in people 75 years of age or older (Adachi *et al.,* 2002; Rodriguez *et al.,* 2006). It has been shown that the serum level of homocysteine is higher in men than in women (Dankner *et al.,* 2004).Some reports have described a significant relationship between higher homocysteine among hypertensives (Dinavahi and Falkner, 2014). The distribution of rs1047891 allele and genotype in the studied population showed the A allele to be the minor allele with an allelic frequency 0.19 which is in conformity with the reported frequency from the Yoruba hapmap database. Homozygous C genotype is the most prevalent genotype in this population which is similar to the report by Silene *et al.,* (2015). The genotype distribution of participants in Ibadan, was consistent withHardy Weinberg equilibrium (pHWE>0.05) which is same as that found in a Caucasian population(Silene *et al.,* 2015).

The SNP (rs1047891) showed no association with hypertension (p>0.05) unlike studies by Hang *et al.,* (2010),Shi *et al.,* (2016) and Villani *et al.,* (2018) who found associations between rs1047891 and hypertension. All genetic models of rs1047891 of CPS1 were adjusted for the confounding effect of sex, age, BMI, SBP and DBP except for the recessive model which showed no result because it was not tangible enough for the software to run due to small sample size. The initial analysis of all parameters was not statistically significant. Although after adjustment, only age under different genetic models was significantly associated with risk association between rs1047891and hypertension under the additive and dominant models suggesting it as a factor that influences the risk predisposition of rs1047891 to hypertension. This is in concordance with Ostrakhovitch and Tabibzadeh, (2019) and Villani *et al.,* (2018), who reported increased risk of hypertension with age.

Linear regression analysis was carried out to show the relationship between the predictor variable (independent variable- age) and the outcome variable (dependent variables- BMI, SBP, DBP) associated with hypertension. There was a statistical significant association with age in both systolic and diastolic blood pressure under all the genetic models. except for body mass index.

Both BMI and SBP had a linear relationship with age; BMI and SBP increases as age increases unlike DBP which showed an inverse relationship with age in all models; DBP decreases as age increases.This agrees with reports by Duprez, (2008) that increase in age results to increase in SBP,

whereas DBP tends to reduce due to age related changes in arterial vasculature, leading to widening of pulse pressure.

For the simulated global data set, the descriptive characteristics showed to have more females as hypertensives than the male participants.A statistically significant mean difference between cases and controls was shownhaving a higher mean age among hypertensives than in normotensives. The systolic and diastolic blood pressure showed to be statistically high among hypertensives. There was also a statistical significant increase in body mass index among hypertensives who showed to be overweight.N4_3 showed a minor allele frequency T to be 0.236, a high homozygous AA genotype frequency (58.6%), n4_4 has a minor allele frequency G to be 0.239, a high homozygous GG genotype frequency (58.5%), m4_0 has a minor allele frequency A to be 0.376, a high homozygous TT genotype frequency (47.6%) while qr2_4 has a minor allele frequency G to be 0.263, a high homozygous AA genotype frequency (54.0%). All four SNPs showed to be in Hardy Weinberg equilibrium (pHWE>0.05) but showed no association with hypertension.Michael *et al.,* (2009)also found no association with hypertension in the polymorphism of rs1061471. As wellas Jamshidi *et al.,* (2018) who found no association with hypertension in the polymorphisms of rs2681472and rs35929607 among the Iranian population studied.

During the initial analysis of these SNPs, all the parameters apart from sex which showed no result, were not statistically significant for all three models but after adjustment, there was a statistical significance in all parameters. Using linear regression, each SNP in the simulated data set showed a statistical significance in all genetic models for BMI, SBP and DBP. There was a linear relationship between age and the outcome variables (BMI and SBP) across the genetic models; increase in age results to increase in BMI and SBP.Although, DBP showed to have an inverse relationship with age in all models for both n4_3 and m4_0, dominant and recessive models for n4_4 and the additive and recessive models for qr2_4; increase in age results to decrease in DBP. A study by Pinto (2007) also confirms an increase in SBP and decrease in DBP with increase in age. This is however in contrast with a report by Wright *et al.*,(2011) who depicted a rise in SBP and DBP with increase in age.

Comparing the findings in both study groups, rs1047891 had only age to be statistically significant influencing the risk predisposition to hypertension while n4_3, n4_4, m4_0 and qr2_4 showed age,SBP, DBP and BMI to be statistically significant in the multivariate analysis using multiple logistic regression. This implies that not only age but SBP, DBP and BMI can also predispose to hypertension. Studies by Shikha *et al.,* (2017) and Hui *et al.,* (2018) also confirm that age, SBP, DBP and BMI are factors that predispose to hypertension. However,in relation to the study population of Ibadan with SNP rs1047891, age showed to be the only variable that was statistically significant to influence predisposition to hypertensiondue to the fact that advancing age is associated with elevated homocysteine levels.This is in line with a study by Ostrakhovitch and Tabibzadeh, (2019)who showed a strong positive association between homocysteine, age, and its risk predisposition to hypertension.

Conclusion

The findings of this study showed no significant association of CPS1(rs1047891) and n4_3, n4_4, m4_0 and qr2_4 with hypertension. Age showed to significantly influence the association with hypertension in the Ibadan population. SBP also significantly influence the association of rs1047891with hypertension which is consistent with other studies done. This implies that both age and SBP are important factors to pay attention to in the development of hypertension.

Recommendations

Based on the result of the study, the following recommendations were made:

1. More research should be conducted in different regions of Nigeria using a large population size.

2. Further investigation should be done involving plasma homocysteine level of every patient for a proper association with hypertension.
3. A reasonable number of SNPs should be initiated into studies for more exploration to affirm the association with hypertension.

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