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Lifestyle and related genetic variations influence susceptibility towards essential hypertension

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Abstract
Essential hypertension is an outcome of complex interaction between genetic and environmental factors. Higher prevalence of hypertension and dyslipidemia in Chandigarh region prompted us to screen genetic (three polymorphisms) as well as lifestyle parameters among individuals from this region, which is known for its advanced and modern culture. Anthropometric measures, lipid profiles along with genetic screening of three polymorphisms namely, rs5186, rs422858 of AT1R, and rs4340 of ACE among individuals from both Hypertensive (HTN) & Normotensive (NTN) groups was performed using auto-analyzer and RFLP techniques. Validation of genotypes was performed by direct sequencing and analysis was done using appropriate statistical tools. NTN group showed significantly lower BMI (p=0.03) than HTN despite comparatively higher percentage of occupational physical inactivity. Mean onset age of hypertension was found to be significantly low among individuals with family history. Among hypertensives, individuals with recent medication (0-3 years) had higher TG and VLDL-C levels than the other group (>3 years). Additionally, allele and genotype analysis revealed higher frequency (p=0.003) of ACE (I) allele among female hypertensive individuals rather than ACE (D) allele. Being the first study on rs422858 (A-214C) among Indians, A allele showed slightly higher frequency for NTN group contrary to the reports from Caucasian population. Present study brings out the higher susceptibility of individuals from Chandigarh region towards EH due to their lifestyle practices and hence warrants lifestyle modification to reduce hypertension related morbidity and mortality

Keywords: AT1R, Anthropometric measures, Essential hypertension, lipid profiles, lifestyle, polymorphism

1. Introduction
Hypertension is known to be an important risk factor for stroke, cardiovascular and other renal disorders which affect more than 1 billion people worldwide [1]. Though such disorders especially cardiovascular diseases (CVDs) are influenced by number of factors; certain factors like age and heritability cannot be influenced hence factor like lifestyle modification is considered as primary practice to maintain normal blood pressure in presence of hypertension. Prevalence and epidemiology of hypertension has reached new heights in India [2] which generates an issue of concern mainly in the urban population [3, 4] where rapid changes in lifestyle pattern are visible. Strict designed diet and reduction in both systolic and diastolic blood pressure reduces the risk of all related symptoms and diseases [5]. Hypertension (high blood pressure) is broadly classified into primary and secondary hypertension, where primary or Essential hypertension (EH) is more prevalent (90-95%) and complicated due to complex interactions of genetic, non-genetic and environmental factors like dietary pattern and routine lifestyle [6, 7]. Lifestyle modifications include both physical activity and dietary pattern. Physical activity, which comprises of active regime like daily exercise/walk/manual labour, lowers the blood pressure as also evident by the lower prevalence of such disorders in rural areas where basic occupation involves agriculture which is labour intensive. Besides this, change in dietary pattern like more of fresh fruits and vegetables with low fat dairy products, high protein content and low sodium intake as per recent guidelines by American Heart Association and European Society of Hypertension show positive influence to maintain normal blood pressure [5]. This is fairly evident from Dietary Approaches to Stop Hypertension (DASH) also, as per guidelines of hypertensive clinics [8]. In addition to healthy diet, alcohol intake and smoking needs discouragement as consumption of alcohol in high amount affects nervous system along with baroreflex receptors to cause alcohol induced hypertension [9]. Increase in blood pressure is also commonly observed in individuals with Dyslipidemia which comprises of altered levels of lipid profile [10].

Studies on hypertensive individuals show increased levels of low density lipoprotein (LDL), total cholesterol (TC), triglycerides (TG) and low level of high density lipoprotein (HDL) in comparison to normotensives**.** [11] Similar observations were made among the lipid profiles of Asian and non-Asian subjects with CVD, further confirming the relativeness between hypertension and CVD pathophysiologies [12]. In recent times even clinicians encourage lifestyle modification to lower down both blood pressure and serum lipid levels as a primary measure before prescribing medicines. Renin Angiotensin System (RAS) still remains the main regulator of blood pressure [13, 14] where regulation by Angiotensin II (Ang II), main biological active peptide is achieved through type 1 (AT1R) or type 2 (AT2R) Angiotensin II receptor. However, AT1R is critical as it mediates most of the functions involved in blood pressure regulation [15, 16] and its blockers are often used as anti-hypertensives. Due to specific focus of this paper on other aspects, readers are suggested to consult other good articles to know more details on AT1R [17-19]. Studies on single nucleotide polymorphisms (SNPs) of AT1R worldwide [20, 21] have elucidated rs5186 (A1166C) as the most widely associated polymorphism with different pathological conditions [22-24]. The positioning of A1166C in the 3'UTR disrupts the binding site of miRNA-155 leading to over expression of AT1R and hence raises blood pressure.[25] Likewise, AT1R promoter polymorphism rs422858 (A-214C) which lies in the binding site of Upstream Stimulatory Factor (USF), is a constitutive part of haplotype shown to increase blood pressure in transgenic mice [26]. Association studies of A1166C with EH from different populations (Table 1) including few from India [33, 34] though do not present very convincing results but they do indicate possibility of SNPs contribution, as also seen in miRNA binding studies for A1166C [25] and transgenic mice in case of A-214C.

Table 1: Allelic frequency of rs5186 (A1166C) in different populations with EH

Population studied	Allelic frequency (Cases) HTN		Allelic frequency (Controls) NTN		Reference
	A	C	A	C	
1) San Luis (Argentina)	0.81	0.19	0.94	0.06	[27]
2) Jordan	0.792	0.208	0.784	0.216	[28]
3) Han	0.967	0.033	0.979	0.021	[29]
4) Tibetan	0.981	0.019	0.939	0.061	
5) Yi	0.945	0.055	0.965	0.035	
6) Odisha (India)	0.908	0.092	0.962	0.038	[30]
7) Serbia	0.72	0.28	0.74	0.26	[31]
8) Nigeria	0.99	0.01	0.99	0.01	[32]
9) North India	0.63	0.37	0.79	0.21	[33]
10) Tamil Nadu (India)	0.616	0.384	0.599	0.401	[34]
11) Brazil	0.744	0.256	0.83	0.17	[35]
12) Ohasama (Japan)	0.91	0.09	0.92	0.08	[36]
13) Poland	0.71	0.29	0.80	0.20	[37]
14) Turkey	0.77	0.23	0.86	0.14	[38]
15) Tamilian (India)	0.95	0.05	0.95	0.05	[39]

Another important enzyme from RAS, Angiotensin converting enzyme (ACE) has major significance in blood pressure regulation as its inhibitors (ACEIs) falls in the primary line of treatment to control hypertension. The gene for ACE is located on chromosome 17 (17q23) with 26 exons and ACE (Ins/Del) polymorphism, comprising of Alu repeat sequence in intron 16, affects plasma and cellular ACE levels with DD genotype causing higher plasma ACE activity [40] resulting in differential Ang II effects. Not only in EH, [41-42] ACE (I/D) polymorphism has been explored in various other conditions [43-45] among Indian population with both positive and negative association. However a difference in the ethnicity of studied samples mars the consensus.

Therefore, Chandigarh residents who are known for their advanced culture and luxurious lifestyle seems to be the right sample set to explore how anthropometric measures vary in this set including individuals with and without essential hypertension. Secondly, is there any allele or haplotype which is predominant and characteristic of this particular region?

2. Materials and Methods

2.1 Recruitment and sample collection

Blood sample (3 ml) was collected from 111 HTN and 104 NTN individuals between age group of 30 to 60 years from Bhai Ghanaiya Ji Institute of Health (Panjab University) and Bharat Vikas Parishad Charitable Medical Centre (BVPCMC), Chandigarh from 2011 till 2016. All the participants were resident of Chandigarh region by either birth or for last 15-20 years. The study was approved by Institutional ethics committee (IEC) of Panjab University and all the participants voluntarily provided their informed written consent for participation.

2.1.1 Inclusion criteria

Diagnosis was based on JNC 7 guidelines where hypertensive group included individuals with systolic blood pressure (SBP)≥140 and diastolic blood pressure (DBP)≥90 mm Hg on two or more visits to clinic after rest of 5-10 min or history of hypertension or undergoing antihypertensive medication [46]. Control group after age and sex match included individuals with SBP≤120 and DBP≤80 mm Hg and no report of hypertension associated diseases like diabetes or other renal disorder and were not on any antihypertensive medication.

2.1.2 Exclusion criteria

Cases with either pregnancy or reports of diabetes, kidney or heart disease that makes them secondary for hypertension were excluded.

2.1.3 Data collection

Clinical details, diet pattern and lifestyle conditions along with family history were taken in a detailed questionnaire for record. Sedentary activity due to occupation/during office hours was recorded and biochemical tests like lipid profiling (TC, TG, HDL-C, LDL-C, VLDL-C), kidney function tests (Urea and creatinine) and Fasting blood sugar (FBS) of individuals from both groups were done by automatic analyzer using commercially available kits at BVPCMC, Chandigarh. Effect of antihypertensive drugs like Calcium channel blockers (CCBs), Beta blockers (BBs) and Angiotensin receptor blockers (ARBs) on serum lipid levels was also analyzed.

2.2 DNA Extraction and Genotyping

Genomic DNA was extracted using Phenol/Chloroform method [47] and PCR-RFLP method was used to genotype

A1166C polymorphism [43] (PCR details available on request). The PCR products (350bp) were digested using DdeI enzyme (NEB) at 37˚C and were electrophoresed on 8% Native Polyacrylamide gel (PAGE). Fragments of 211bp and 139bp indicated presence of CC genotype (Fig.1)

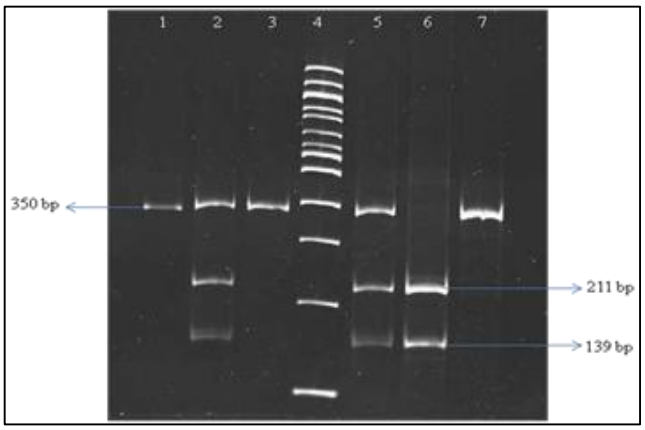

Fig 1: PCR-RFLP profile of rs5186 on 8% Native PAGE
Lane 1: PCR product, Lane 2&5: AC, Lane 3&7: AA, Lane 4: 100 bp marker, Lane 6: CC

SNP A-214C was similarly amplified using 5' TCCGCAGGAAATGATACTCCTG 3' (forw) and 5' TTTATAGTGAGGGGCGTTGCTG 3' (rev) primers, (PCR conditions available on request) and PCR products (256bp) were digested with BstNI enzyme (NEB) at 60˚C. The digested PCR products on 8% Native PAGE yielded fragments of 182bp and 74bp (AA genotype) and 256bp (CC genotype) (Fig. 2).

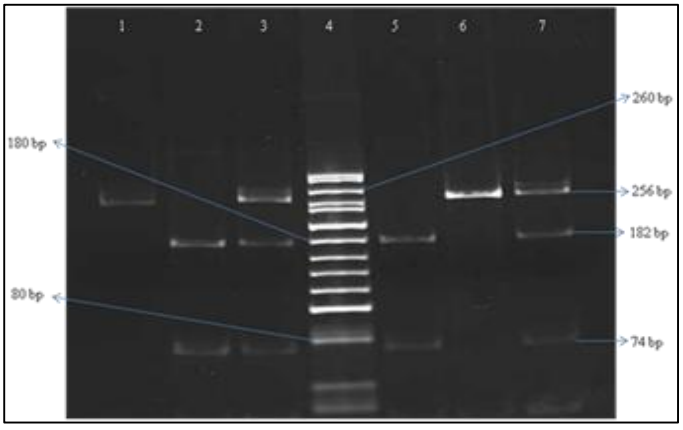

Fig 2: Digestion profile of A-214C polymorphism on 8% Native PAGE
Lane 1: PCR product, Lane 2&5: AA, Lane 3&7: AC, Lane 4: 20 bp ladder, Lane 6: CC

Direct sequencing of purified PCR products for both A1166C (Fig.3) and A-214C polymorphism (Fig.4) was done to validate the RFLP profiles.

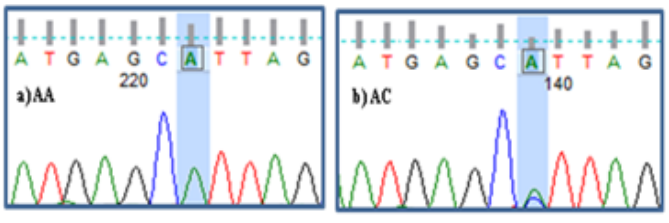

Fig 3: Sequencing results of rs5186

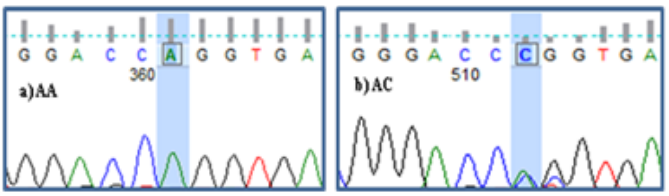

Fig 4: Sequencing results of A-214C polymorphism

ACE (I/D) polymorphism was screened by using 5'CTGGAGACCACTCCCATCCTTTCT3' (forw.) and 5'GATGTCGCCATCACATTCGTCAGAT3' (rev.) primers to generate 490 and 190 bp amplicon depicting I/I, D/D or I/D genotype (Fig.5) (PCR details available on request).

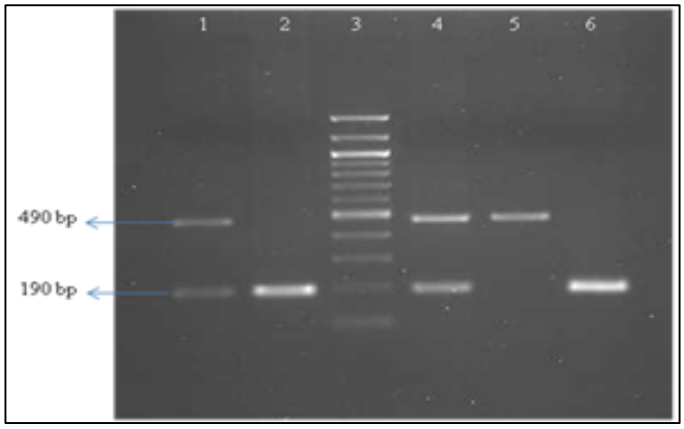

Fig 5: PCR amplified product of *ACE*
Lane 1&4: ID, Lane 2&6: DD, Lane 3: 100 bp marker, Lane 5: II

2.3 Statistical Analysis

Genotype frequencies were calculated by gene counting method. Chi square test (http://graphpad.com/quickcalcs/PValue1.cfm) was performed to check Hardy-Weinberg distribution among study groups. Odds ratio for allelic and genotypic frequencies in both HTN and NTN as total and further, on basis of gender were obtained by online tool (http://www.medcalc.org/calc/odds_ratio.php). Difference in allelic and genotypic frequencies was analyzed using same tool on segregation of population into family history and onset age. P value less than 0.05 was considered to be significant. Difference of multiple variables between both groups was calculated by unpaired t-test using GraphPad software (http://graphpad.com/quickcalcs/ttest1/) that also provides mean and standard deviation of each individual group.

3. Results

A total of 215 individuals for A1166C and A-214C polymorphism of AT1R and 211 individuals for ACE (I/D) were screened (readable profiles were not obtained in remaining samples). Both groups (HTN and NTN) were in Hardy-Weinberg equilibrium ($p>0.05$) for each of the studied SNP. Allele and genotype frequencies among HTN and NTN groups as total and on further division based on gender, were analyzed (Table 2, 3). Additionally, analysis on hypertensives, segregated on the basis of positive and negative family history, did not show significant difference (Table 4). However, segregation on basis of onset age of hypertension; below 40 years of age (early onset) and at the age of 40 or later (late onset) showed difference in genotypic frequencies (AC) of A1166C and (DD) of ACE among these

groups (Table 5). Biochemical tests including lipid profile (LDL-C, HDL-C, TC, TG), fasting sugar, urea and serum creatinine levels showed no statistically significant difference between different groups except SBP and DBP readings. SBP and DBP was observed as per expectations significantly (p=0.0001) lower among NTN (Table 6). Along with this, mean onset age of hypertension also showed significant difference among familial and sporadic group (40.1±5.8 vs 45.6±5.9, p=0.03) with onset of hypertension much earlier in familial group. No significant differences were found in percentage of factors like alcohol consumers, smokers, non-veg diet and sedentary lifestyle among HTN and NTN. However, significant difference was observed in BMI (27.12±4.2 vs 25.06±2.9, p=0.03) as shown in Table 6.

Table 2: Genotype and allele frequencies of A1166C and A-214C polymorphisms of AT1R

Genotypes/Alleles	Total (%)		Males (%)		Females (%)	
	HTN (n=111)	NTN (n=104)	HTN (n=59)	NTN (56)	HTN (n=52)	NTN (n=48)
A1166C						
AA	101 (90.9)	91 (87.5)	54 (91.5)	48 (85.7)	47 (90.4)	43 (89.6)
AC	10 (9.1)	13 (12.5)	5 (8.5)	8 (14.3)	5 (9.6)	5 (10.4)
CC	0	0	0	0	0	0
OR (95% CI); *p* value*	0.6931 (0.2889-1.6572); *p*=0.41		0.5556 (0.1702-1.8136); *p*=0.33		0.9149 (0.2476-3.3799); *p*=0.89	
A	212 (95.5)	195 (93.7)	113 (95.8)	104 (92.9)	99 (95.2)	91 (94.8)
C	10 (4.5)	13 (6.3)	5 (4.2)	8 (7.1)	5 (4.8)	5 (5.2)
OR (95% CI); *p* value	0.7075 (0.3033-1.6505); *p*=0.42 (A vs C)		0.5752 (0.1824-1.8143); *p*=0.35 (A vs C)		0.9192 (0.2577-3.2792); *p*=0.90 (A vs C)	
A-214C						
AA	76 (68.5)	72 (69.2)	44 (74.6)	37 (66.1)	32 (61.6)	35 (72.9)
AC	33 (29.7)	30 (28.9)	15 (25.4)	18 (32.1)	18 (34.6)	12 (25)
CC	2 (1.8)	2 (1.9)	0	1 (1.8)	2 (3.8)	1 (2.1)
OR (95% CI); *p* value*	0.9651 (0.5416-1.7197); *p*=0.90		1.5063 (0.6729-3.3721); *p*=0.32		0.5943 (0.2548-1.3862); *p*=0.23	
A	185 (83.3)	174 (83.7)	103 (87.3)	92 (82.1)	82 (78.8)	82 (85.4)
C	37 (16.7)	34 (16.3)	15 (12.7)	20 (17.9)	22 (21.2)	14 (14.6)
OR (95% CI); *p* value	0.9770 (0.5869-1.6263); *p*=0.93 (A vs C)		1.4928 (0.7222-3.0856); *p*=0.28 (A vs C)		0.6364 (0.3046-1.3294); *p*=0.23 (A vs C)	

OR Odds Ratio, 95% CI 95% Confidence level, *Value of genotypes (AA vs AC and CC)

Table 3: Genotype and allele frequencies of ACE (I/D) polymorphism

Genotypes/Alleles	Total (%)		Males (%)		Females (%)	
	HTN (n=111)	NTN (n=100)	HTN (n=59)	NTN (n=54)	HTN (n=52)	NTN (n=46)
ACE (I/D)						
II	33 (29.7)	25 (25)	16 (27.2)	18 (33.3)	17 (32.7)	7 (15.2)
ID	62 (55.9)	49 (49)	31 (52.5)	25 (46.3)	31 (59.6)	24 (52.2)
DD	16 (14.4)	26(26)	12 (20.3)	11 (20.4)	4 (7.7)	15 (32.6)
OR (95% CI); *p* value	0.4662 (0.2072-1.0492); *p*=0.07 (DD vs II) 0.9586 (0.5051-1.8191); *p*=0.90 (ID vs II) 0.4864 (0.2351-1.0060); *p*=0.05 (DD vs ID) 0.7879 (0.4286-1.4482); *p*=0.44 (ID+DD vs II)		1.2273 (0.4253-3.5412); *p*=0.70 (DD vs II) 1.3950 (0.5932-3.2803); *p*=0.45 (ID vs II) 0.8798 (0.3325-2.3280); *p*=0.80 (DD vs ID) 1.3438 (0.6002-3.0085); *p*=0.47 (ID+DD vs II)		0.1098 (0.0268-0.4503); **p*=0.002 (DD vs II) 0.5319 (0.1901-1.4882); *p*=0.23 (ID vs II) 0.2065 (0.0606-0.7028); **p*=0.01 (DD vs ID) 0.3695 (0.1371-0.9960); *p*=0.05 (ID+DD vs II)	
I	128 (57.7)	99 (49.5)	63 (53.4)	61 (56.5)	65 (62.5)	38 (41.3)
D	94 (42.3)	101 (50.5)	55 (46.6)	47 (43.5)	39 (37.5)	54 (58.7)
OR (95% CI); *p* value	0.7198 (0.4901-1.0572); *p*=0.09 (I vs D)		1.1331 (0.6703-1.9152); *p*=0.64 (I vs D)		0.4222 (0.2377-0.7498); **p*=0.003 (I vs D)	

OR Odds Ratio, 95% CI 95% Confidence level, *Significant p value<0.05

Table 4: Distribution of allele and genotype of A1166C, A-214C and ACE (I/D) polymorphism in HTN group subdivided on the basis of family history

Genotypes/Alleles	HTN (%)		
	Family history (n=44)	No family history (n=67)	OR (95% CI); *p* value*
A1166C			
AA	39 (88.6)	62 (92.5)	1.5897 (0.4321-5.8492); *p*=0.49
AC	5 (11.4)	5 (7.5)	
CC	0	0	
A	83 (94.3)	129 (96.3)	1.5542 (0.4365-5.5339); *p*=0.50
C	5 (5.7)	5 (3.7)	
A-214C			
AA	33 (75)	43 (64.2)	1.6744 (0.7188-3.9004); *p*=0.23
AC	10 (22.7)	23 (34.3)	
CC	1 (2.3)	1 (1.5)	
A	76 (86.4)	109 (81.3)	1.4526 (0.6875-3.0692); *p*=0.33
C	12 (13.6)	25 (18.7)	
ACE (I/D)			
II	14 (31.8)	19 (28.4)	0.8482 (0.3708-1.9403); *p*=0.70
ID	27 (61.4)	35 (52.2)	
DD	3 (6.8)	13 (19.4)	
I	55 (62.5)	73 (54.5)	0.7180 (0.4145-1.2438); *p*=0.24
D	33 (37.5)	61 (45.5)	

OR Odds Ratio, 95% CI 95% Confidence level, *Value of genotypes (AA vs AC and CC) (II vs ID and DD)

Table 5: Distribution of allele and genotype of A1166C, A-214C and ACE (I/D) polymorphism in HTN group sub divided on the basis of onset age

Genotypes/Alleles	HTN (%)		
	Early onset (n=33)	Late onset (n=78)	OR (95% CI); *p* value*
A1166C			
AA	28 (84.8)	73 (93.6)	2.6071 (0.7007-9.7008); *p*=0.15
AC	5 (15.2)	5 (6.4)	
CC	0	0	
A	61 (92.4)	151 (96.8)	2.4754 (0.6919-8.8568); *p*=0.16
C	5 (7.6)	5 (3.2)	
A-214C			
AA	23 (69.7)	53 (67.9)	1.0849 (0.4493-2.6200); *p*=0.86
AC	10 (30.3)	23 (29.5)	
CC	0	2 (2.6)	
A	56 (84.8)	129 (82.7)	1.1721 (0.5317-2.5838); *p*=0.69
C	10 (15.2)	27 (17.3)	
ACE (I/D)			
II	10 (30.3)	23 (29.5)	0.9618 (0.3959-2.3368); *p*=0.93
ID	20 (60.6)	42 (53.8)	
DD	3 (9.1)	13 (16.7)	
I	40 (60.6)	88 (56.4)	0.8412 (0.4679-1.5121); *p*=0.56
D	26 (39.4)	68 (43.6)	

OR Odds Ratio, 95% CI 95% Confidence level, *Value of genotypes (AA vs AC and CC) (II vs ID and DD)

Table 6: Comparative analysis of various parameters of HTN and NTN subjects

Parameters	Cases (HTN) n=111	Controls (NTN) n=104	*p* Value
Sex (M/F)	59/52	56/48	
Alcohol consumption, AC (%)	26	29	0.66
Smoking (%)	12	13	0.77
Sedentary activity, SA (%)	53	61	0.42
Non-Veg diet, NV (%)	39	52	0.23
AC+NV (%)	18	26	0.34
Age (years)	43.3±6.8	44.2±9.4	0.42
BMI (Kg/ m^2)	27.12±4.2	25.06±2.9	0.03*
SBP (mmHg)	139.9±10.6	125.8±5.0	0.0001*
DBP (mmHg)	86.3±7.7	80.2±4.7	0.0001*
TG (mg/dl)	143.7±25.3	139.8±38.0	0.70
TC (mg/dl)	170.5±28.6	177.5±30.6	0.47
HDL-C (mg/dl)	46.7±7.3	47.2±11.0	0.86
LDL-C (mg/dl)	98.8±25.9	102.1±36.2	0.73
VLDL-C (mg/dl)	29.4±6.0	30.7±9.6	0.58
FBS (mg/dl)	90.8±11.3	86.2±9.1	0.14
Urea (mg/dl)	26.1±6.3	25.8±5.3	0.87
Uric acid (mg/dl)	4.5±1.2	5.2±1.3	0.06
Creatinine (mg/dl)	0.89±0.2	0.99±0.12	0.05

Continuous variables are represented as Mean ± Standard Deviation and frequency data as percentage

BMI Body Mass Index, *SBP* Systolic Blood Pressure, *DBP* Diastolic blood Pressure, *TG* Triglycerides, *TC* Total Cholesterol, *FBS* Fasting Blood Sugar, *HDL-C* High density Lipoprotein cholesterol, *LDL-C* Low density lipoprotein cholesterol, *VLDL-C* Very low density lipoprotein cholesterol *Significant *p value<0.05*

4. Discussion

Chandigarh, the union territory is one of the best examples of planned city in India with modern architecture and increased accessibility to educational, cultural and medical facilities. This led to rapid growth of population in the last decade from 2001 to 2011with an average growth rate of 4% every year and its population has reached the mark of 1,165,565 in 2016 as a result of immigration of people from nearby regions. Chandigarh residents in general are known for luxurious, carefree lifestyle including high intake of alcohol, non-veg diet and more adapted to automated processes leading to more of sedentary activities. These factors are known to increase the risk for obesity, hypertension and other cardiovascular disorders. [48-49] This is supported by the data of Bhansali *et al*. [50] where they reported hypertension to be 31% prevalent in urban residents of Chandigarh. Along with this, increased prevalence of dyslipidemia was found among 87% of the residents of Chandigarh. Such high prevalence of hypertension along with dyslipidemia in this region prompted us to screen genetic as well as lifestyle factors for hypertension in Chandigarh.

4.1 rs422858 (A-214C polymorphism)

SNP A-214C which was studied with other promoter polymorphisms as a haplotype in hypertension [51] and myocardial infarction [52] showed positive association with hypertension among Caucasians and with raised blood pressure as a haplotype in transgenic mice [26]. Minor allele C of this SNP was associated with extreme old age in Italian cohort [53] as it reduced AT1R receptor expression levels which provided protection against Ang II mediated stimulation of mitochondrial reactive oxygen species (ROS) resulting in extended lifespan. But no report of this SNP on

Indian population is available as per literature, making this the very first report from India. Although no significant statistical difference between frequency of A allele among NTN (0.837) and HTN (0.833) was observed but gender based segregation revealed slightly higher frequency in HTN males (0.873) and lower in HTN females (0.788) in comparison to respective NTN group (Table 2). Notably, the frequency of allele A in normal individuals was much higher (0.837) than Caucasians (0.74), [26] indicative of population differences.

4.2 rs5186 (A1166C polymorphism)

Another polymorphism A1166C from 3'UTR is the most commonly studied polymorphism ever since Bonnardeaux *et al*. [54] reported a higher frequency of C^{1166} allele in HTN group. Like other SNPs, A1166C also presents mix of positive and negative association outcomes [27, 32, 35, 36, 38]. Though C^{1166} allele is prominent among families with positive history, [28, 55] it also shows gender specific association with hypertension [29, 31, 56]. Similar studies from India [30, 33, 34, 57] are very few but analysis of these studies reveal differences in regional population, selection criteria and age as main factors for contradictions in outcomes. This pilot study on A1166C shows no significant association (Table 2), similar to another report from South Indian population [34, 39] but the allelic frequency of C^{1166} among NTN (0.063 in Table 2) was different from the reported frequency in the South Indian population (0.40) [34] and much similar (0.06) to the frequency reported by Kaur *et al*. [43] However another study from north Indian population reported positive association with hypertension plausibly due to the fact that the samples collected in that study [33] were from one of the premier medical institute of India which covers most of the northern region of India whereas the samples in this study represents Chandigarh region specifically.

4.3 rs4340 (ACE (I/D) polymorphism)

ACE (I/D) has been studied in various diseases [40, 44, 45, 58] including hypertension where DD genotype was found positively associated with hypertension, [59, 60] but studies with negative association also exist [61]. Surprisingly, studies on north Indian subjects found allele I to be associated with EH [62] and DD genotype to be more common among controls [42] which is in contradiction with other reports. [59, 60] We also report significant difference (0.003, Table 3) between both alleles in females after gender based segregation where allele I was more common in hypertension group and D allele among controls. However this difference needs further validation with larger sample size.

4.4 Lipid Profiles

Statistical calculations on mean serum levels (mg/dl) among studied samples revealed surprisingly slightly higher levels of LDL-C (102.1±36.2), HDL-C (47.2±11.0) and TC (177.5±30.6) among normotensives (NTNs) rather than HTNs but TG (143.7±25.3) was higher among HTNs as per speculations (Table 6). Further analysis into the lifestyle (daily routine and diet) of these NTN individuals put forth the reasons for this unexpected outcome. Among NTNs, we observed higher percentage of individuals with sedentary routine (61%) and non-veg diet (52%) though the blood pressure (BP) readings were normal along with other anthropometric measures at the time of sample collection. But as we know that essential hypertension is a silent killer and it does not show any major symptom in early stages and demands routine monitoring, so these measures indicate that NTNs though presently do not fall into the inclusion criteria of HTN but they may be at risk. Status of lipid profiles will generate awareness among these individuals about current situation and motivate them to modify their routine to prevent any diseased situation in future. This result gets supported by state wide survey in Punjab for non-communicable diseases where prevalence of hypertension, hypertriglyceridemia and hypercholesterolemia came out to be 40%, 22% and 16% respectively [63]. However, lower mean serum lipid level in HTNs could be due to ongoing antihypertensive medication as antihypertensive drugs show differential effect on lipid levels [64]. This was further supported from the fact that individuals who started medication recently (0-3 years) had higher TG (145±35.8 vs 136.6±20.1) and VLDL-C (31.3±9.0 vs 27.4±4.0) and almost comparable TC (173.4±33.9 vs 173.6±25.5) and HDL-C (44±5.5 vs 44.5±5.5) levels when compared with those who were on medication for longer time period (>3 years). TC (179.5±29.7 vs 166.6±25.6) and LDL-C (106.8±30.7 vs 95.9±17.9) levels were higher in familial group in comparison to sporadic individuals (no family history). In an identical study elevated blood pressure and raised serum lipid levels were reported in offspring of hypertensive parents in comparison to normotensive parents. [65] Category wise effect of antihypertensive drugs on serum lipid levels resulted in substantial reduction in TG (128.1±22.4 vs 151.7±39.8), LDL-C (89.3±26.7 vs 94.8±37.6) and VLDL-C (25.6±4.7 vs 34±9.9) levels using calcium channel blockers (eg. Amlodipine) and beta blockers (eg. Atenolol, Metoprolol) either alone or in combination when compared with ARBs (eg. Telmisartan, Losartan). Similar effect of ACEIs and BBs on blood glucose, total cholesterol and triglycerides levels in antihypertensive combination therapies was seen in literature [66].

4.5 Lifestyle factors

Higher percentage of individuals with occupational sedentary activity (61% vs 53%) and non-vegetarian diet (52% vs 39%) was observed in NTNs than HTNs but were still normal can be attributed to the fact that most of these individuals had the habit of morning/evening walk or running. Additionally, most of these individuals were not regular or passionate consumers of non-veg diet, therefore, rare non-veg dietary pattern might have worked as a protein supplement similar to what is provided in DASH diet (Dietary Approaches to Stop Hypertension- includes mild non-veg diet) to control blood pressure in individuals with hypertension & non-veg dietary pattern [67]. In the absence of any significant statistical difference (p=0.23) role of non-veg diet (Table 6) towards development of EH cannot be concluded. Though maternal low protein diet (MLPD) during pregnancy in mice dams have been reported to cause epigenetic changes and differential expression in fetal brain RAS [68]. Further to this study, MLPD during gestation in mice caused development of hypertension in sex specific manner with female offsprings having severe hypertension. [69] Results on mice study made us to hypothesize that a minimum level of protein supplement is helpful in maintaining normal blood pressure. Thus, practices of physical exercises, like walk/run and rare non-veg or proteinaceous diet might have prevented development of high blood pressure in NTN group of this study. This is further evident from significant difference in BMI readings of HTN and NTN group (27.12±4.2 vs 25.06±2.9, p=0.03). But this conclusion warrants more studies with larger sample size.

The authors accept the fact that sample size is very less but seeing the variability in allelic frequencies [33, 34, 39, 43], present study documents the allelic and genotypic frequencies for both SNPs of AT1R along with ACE (I/D) polymorphism in individuals specifically from Chandigarh region along with effects of diet and lifestyle pattern on lipid profile and blood pressure. This data will be quite informative in the future epigenetic studies to present the complete characterization of susceptible factors in these individuals and make them aware about the possible benefits of change in lifestyle pattern to lead a healthy life.

5. Conclusion

We performed the first study on rs422858 (A-214C) polymorphism of AT1R in Indian population. Present study although represents a small scale investigation into the influences of the lifestyle factors and related genetic factors with respect to essential hypertension, it does bring out the higher susceptibility of studied individuals towards EH and thereby to downstream diseases and disorders, further substantiating the requirement to modify our lifestyle, if we really wish to reduce the ever growing impacts of non-communicable diseases (NCDs) in this modern society.

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