JEPIBIO
Journal of Epidemiology and Infection Biology

Research Article

Diet Gene Interaction Studies for Angiotensin Converting Enzyme Insertion/Deletion Polymorphism in Punjabi Pakistani Population

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Date of Receiving: 30/09/2024
Date of Acceptance 15/10/2024
Date of Publishing 10/12/2024

ABSTRACT Hypertension is most widely distributed chronic disease in all over the world and a modifiable risk factor for cardiovascular diseases and morbidity. Renin-Angiotensin-Aldosterone Pathway is major hypertension regulating pathway. Angiotensin Converting Enzyme (ACE) gene showing different genotypes has different susceptibility towards hypertension. The primary aim of this study was to explore how diet and genetic factors interact with hypertension and ACE polymorphism. A total of 116 individuals comprised of hypertensive cases (n=77) and normotensive controls (n=39) were included. Additionally, to understand association of diet, a semi structured questionnaire was used to collect the data. The blood samples of all individuals were analyzed for ACE gene I/D polymorphism. Diet data were also analyzed for intake of sodium, magnesium, calcium, and potassium. Statistical analysis showed association between hypertension and diet, but the polymorphism was not found to be playing role in hypertension. It was revealed that hypertension was associated with gender and smoking habits as 31% of hypertensive patients were smokers while only 17% smokers found in normotensives. Gene diet interaction of hypertension and ACE gene I/D polymorphism was found to be not affected by high sodium salt concentration in their diet. Persons taking low magnesium, calcium and potassium in diet were found more prone to hypertension, irrespective of I/D polymorphism of ACE gene. It is suggested that other genetic and environmental influences might hold more prominent roles in the onset and management of hypertension. To validate and build upon these conclusions, further research with larger participant pools and more diverse populations is necessary.

KEYWORDS Hypertension, Angiotensin converting enzyme, ACE gene, I/D polymorphism

Introduction

Hypertension, a globally prevalent condition, disproportionately affects individuals aged 20 to 65 years, with its onset typically occurring after the age of 40 (Tain and Hsu, 2022; Tang *et al*, 2022). However, younger individuals may also develop hypertension due to underlying health conditions or genetic predisposition (Xu *et al*, 2021). It is already determined that offspring of hypertensive parents are more likely to inherit the condition which indicates the hereditary component (Doaei and Gholamalizadeh, 2014). Additionally, environmental factors also play a critical role in the development of hypertension. In Pakistan, hypertension accounts for 1.5% of all deaths which forms approximately 20 cases per 100,000 individuals (Almas *et al*, 2023).

Hypertension development is based on a complex interaction of genetic, lifestyle, and environmental factors. Lifestyle elements such as obesity, unhealthy diets, lack of exercise, and poor medication compliance significantly contribute in hypertension (Doaei and Gholamalizadeh, 2014; Shaikh *et al*, 2014; Hussain *et al*, 2018; Almas *et al*, 2023). Additionally, smoking has been identified as a modifiable risk factor in case of elevated blood pressure (Ain and Regmi, 2015; Samadian *et al*, 2016). The type and duration of smoking further amplify this risk. The socio-economic conditions in Pakistan exacerbate the burden of hypertension. With these factors, hypertension has emerged as the leading contributor to morbidity and mortality in Pakistan. Therefore, urgent public health intervention is a dire need to counter this problem.

The renin-angiotensin-aldosterone system (RAAS) is a hormonal pathway which plays crucial role in regulating blood pressure and fluid balance (Bernstein *et al*, 2014). The

To cite this article: Noureen, F., H. Saeed, Q.-U.-A. Haider, and M. Hussain. (2024). *Diet Gene Interaction studies for Angiotensin Converting Enzyme Insertion/Deletion Polymorphism in Punjabi Pakistani Population*. Journal of Epidemiology and Infection Biology 1(2):7-13.

Angiotensin-Converting Enzyme (ACE) is a central part of this system. It catalyzes the conversion of Angiotensin I to the potent vasoconstrictor Angiotensin II. Angiotensin II constricts the blood vessels which increases sodium retention, and raises blood pressure. It also explains the key role of ACE in hypertension. The ACE gene is located on the 17th chromosome. It exhibits an insertion/deletion (I/D) polymorphism that significantly influences individual susceptibility to hypertension (Shaikh *et al*, 2014; Rana *et al*, 2018). This polymorphism, particularly the DD genotype, has been linked to several conditions such as cardiovascular diseases and diabetes.

The interaction between genetic variations in the ACE gene and dietary factors like sodium intake further highlights the complexity of hypertension management. Daily dietary habits and lifestyle in Pakistan could be associated with hypertension development. The current study aims to investigate the interaction between diet and the ACE I/D polymorphism in the context of hypertension. The study also seeks to determine how the combination of sodium intake and ACE gene variants influences susceptibility to hypertension in the Punjabi Pakistani population. These findings are intended to provide insights into personalized approaches for preventing and managing hypertension based on genetic and dietary factors.

Materials and Methods

Study Design

A case-control study was conducted to understand the association between diet and the ACE I/D polymorphism. Additionally, a semi-structured questionnaire was disseminated to collect information regarding anthropometry, demography, medical and family history, medication usage, and dietary habits for all participants. A total of 116 individuals were selected from patient records of different hospitals in Sargodha, Pakistan. Anthropometric variables were included such as body weight, body mass index, and height, while demographic details were included such as age, gender, marital status, lifestyle, and location. Blood pressure measurements were taken while the subjects were in a sitting position, using an inflatable cuff placed on their upper arm.

Inclusion and Exclusion Criteria

Only those individuals were included as cases (n=77) in the study who were older than 20 years, had positive history of hypertension, and were taking salt regularly in diet. Those individuals were considered as controls or normotensive (n=39) who never had any history of hypertension. Additionally, inclusion criteria also included the written consent provided by participants. On the other hand, individuals with age less than 20 years, pregnant or nursing mothers, and chronic diseases including Hepatitis and HIV, cancer were excluded from the study.

Collection of Blood Samples and DNA Extraction

Blood samples of all the participants were collected using established protocols. Peripheral blood ranging from three millimeters to five millimeters (3ml-5ml) were obtained from each individual and stored at -80ºC in sterile vials containing EDTA for subsequent analysis. The samples were tagged to prevent any possible mixing cases and controls, and each participant's blood sample and questionnaire were given a unique based labeling. DNA of both cases and control samples were extracted following organic Phenol-Chloroform-Iso-amyl Alcohol (PCI) method and was stored at -20ºC.

Polymerase Chain Reaction

The desired fragment of DNA was amplified following standard PCR protocol. Reported set of forward and reverse primers (ACEF1 ;ACER1) were used (Shaikh *et al*, 2014). The amplicon on gel was visualized with a UV-illuminator and photographed on a Gel Doc (Cleaver Scientific). ACE I/D polymorphism was confirmed based on the product size of amplified PCR products. There were three bands visible: 190bp (genotype DD), 490bp (genotype II) or both 490 bp and 190 bp bands (genotype ID).

Data Analysis

For quantitative variables, means and standard deviation were calculated, whereas for qualitative variables, frequency was calculated. To compare quantitative variables, the One-way ANOVA and independent Student t-test were employed. The qualitative variables, on the other hand, were compared using Yate's adjusted chi square (x2) test. The age, blood pressure, and biochemical parameters of the control, type I diabetes mellitus, and type I diabetic nephropathy disease groups were differentiated using a one-way ANOVA. The Statistical Package for the Social Sciences (SPSS) was used (SPSS 25.0, Chicago, II., USA). $P<0.05$ was used as the significance level.

Results

Reliability Analysis for Measuring Instrument

A total of 116 blood samples were collected from both cases (n=77) and controls (n=39). Comparing various factors revealed significant association with cases and controls. It was found that females were more susceptible to hypertension then males. Further it was found that dietary factors such as salt intake were significantly higher in the normotensive group, while magnesium, calcium, and potassium levels were significantly lower compared to the normotensive group (Table 1).

Demographics Characteristics of patients with Atopic Dermatitis

Genotyping of ACE I/D was accomplished using an internally developed PCR assay method. Amplified product's visualization under UV light revealed a specific amplification of 490 bp and 190 bp product sizes (Fig. 1). Among total 116 samples, heterozygous (ID) were found higher (n=37) while homozygous (II and DD) were found less frequently (n=34 and n=23, respectively). On the other hand, unclassified polymorphs (n=22) were also determined.

Table 1: Characterization of anthropometric, clinical, and dietary intake according to hypertension diagnosis.

Parameters		**Normotensive (n=39)**	**Hypertensive (n=77)**	**p-value**
Gender	Male	30 (77%)	38 (49%)	0.004
	Female	9 (23%)	39 (51%)	
Smoking status	Yes	6 (17%)	24 (31.16%)	0.042
	No	33 (83%)	53(68.84%)	
Family history	Yes	37(95%)	42 (55%)	<0.001
	No	2 (5%) 0	35 (45%)	
Salt intake	High	34 (87%)	25 (32%)	<0.001
	Low	5 (13%)	45 (58%)	
Age		38±8	55±13	<0.001
BMI		24±4	28±6	0.005
SBP		123±7	136±23	0.001
DBP		80±2	85±13	0.013
Magnesium		234±39	192±67	<0.001
Calcium		198±31	167±60	0.003
Potassium		4047±673	3449±1297	0.008

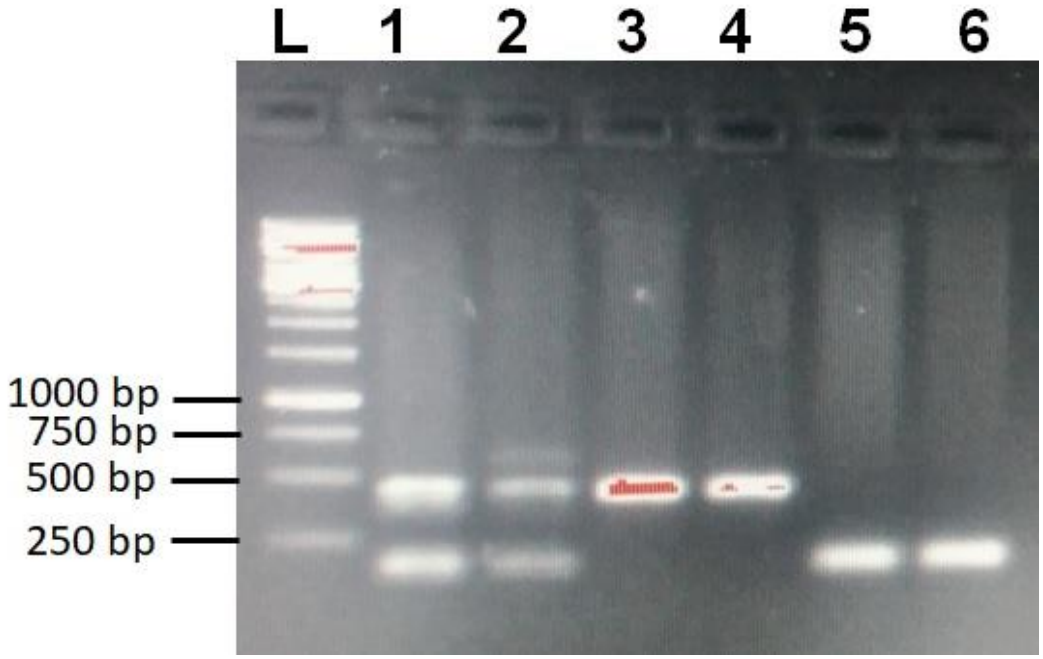

Fig. 1: Genotyping of ACE polymorphism by PCR. In this figure, lane L shows 1 kb Ladder, while lane 1 & 2 shows ID (heterozygous), lane 3 & 4 is showing 490 bp band size which is homozygous II genotype, and lane 5 & 6 are showing 190 bp band size which is homozygous DD genotype.

Association of ACE genotypes and overall individual characteristics

Association of demographic and clinical characteristics with three genotype groups (n=94) revealed important insights (Table 2). The proportion of males and females was similar across all genotypes, with no significant difference (p=0.99). Most participants in all groups had hypertension, with no notable difference among genotypes (p=0.58). Obesity status and smoking habits showed a non-significant distribution. Family history of hypertension was more common in the II and ID groups than in the DD group, nearing statistical significance (p=0.06). High salt intake was more prevalent in the ID and DD groups compared to II, but this difference was not significant (p=0.18). Mean age, BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were comparable among the groups. Serum magnesium, calcium, and potassium levels also showed no significant differences between genotypes. Overall, no parameter reached statistical significance, indicating homogeneity among the groups.

Table 2. Effect of ACE I/D polymorphism on anthropometric, clinical, and dietary factors. Incomplete rows were removed from data and only complete records were analyzed**.**

Parameters		**II (n=34)**	**ID (n=37)**	**DD (n=23)**	**Significance**
Gender	Male	19 (56%)	21 (57%)	13 (57%)	0.99
	Female	15 (44%)	16 (43%)	10 (43%)	
Hypertension	Yes	19 (56%)	21 (57%)	13 (57%)	>0.9
	No	15 (44%)	16 (43%)	10 (43%)	
Obesity status	Normal	4 (12%)	3 (08%)	6 (26%)	0.61
	Overweight	11(32%)	10 (27%)	6 (26%)	
	Obese	5 (15%)	3 (8%)	3 (13%)	
Smoking status	Yes	9 (27%)	9 (24%)	8 (35%)	0.76
	No	22 (64%)	26 (70%)	15 (65%)	
Family history	Yes	20 (59%)	20 (54%)	19 (83%)	0.06
	No	14 (41%)	17 (46%)	4 (17%)	
Salt intake	High	14 (41%)	18 (49%)	12 (52%)	0.18
	Low	20 (59%)	19 (51%)	11 (48%)	
Age		53±13	50±13	50±13	0.58
BMI		28±6	27±5	25±5	0.34
SBP		134±21	135±26	130±17	0.68
DBP		83±15	84±10	85±10	0.94
Magnesium		190±71	207±55	191±62	0.48
Calcium		164±64	178±49	165±57	0.52
Potassium		3369±1360	3683±1048	3386±1204	0.49

Association of ACE and hypertensive individual characteristics

The findings of demographic, clinical, and biochemical parameters association with ACE genotype groups in hypertensive individuals are shown in Table 3. Gender distribution, obesity status, categorized as normal, overweight, and obese, showed no notable variation between groups (p=0.99). On the other hand, Blood pressure (BP) control was also comparable across genotypes (p=0.97). Smoking status did not significantly differ among groups, with similar proportions of smokers and non-smokers (p=0.86). Family history of hypertension was more common in the ID group (57%) compared to the II (45%) and DD (22%) groups, nearing significance (p=0.05). High salt intake is slightly more frequent in the ID and DD groups compared to II, though not statistically significant (p=0.25). Mean age, BMI, and serum levels of magnesium, calcium, and potassium are consistent across all groups, with no significant differences noted.

ACE polymorphism and salt intake interaction

Blood pressure control across genotypes under high and low salt intake revealed different patterns (Fig. 2). Under high salt intake, poor blood pressure control was more frequent in the ID genotype. Good blood pressure control was more common in the II genotype with low salt intake. On the other hand, calcium, magnesium, and potassium levels had little variation across genotypes. Calcium levels were higher in the ID genotype under high salt intake. Magnesium levels showed minimal variation across genotypes and salt intake levels. While Potassium levels were consistently higher in the ID genotype for both salt conditions.

Table 3: Effect of ACE I/D polymorphism on anthropometric, clinical, and dietary factors in hypertensive patients

Parameters		II (n=29)	ID (n=28)	DD (n=18)	Significance
Gender	Male	15 (52%)	13 (46%)	10 (56%)	0.82
	Female	14 (48%)	15 (54%)	8 (44%)	
Obesity status	Normal	2 (7%)	1 (4%)	2 (11%)	0.99
	Overweight	8 (28%)	4 (14%)	5 (28%)	
	Obese	5 (17%)	2 (7%)	3 (17%)	
BP control	Poor	14 (48%)	12 (43%)	8 (44%)	0.97
	Good	15 (52%)	14 (50%)	10 (56%)	
Smoking status	Yes	9 (31%)	8 (29%)	7 (39%)	0.86
	No	20(69 %)	20(71%)	11(41%)	
Family history	Yes	13 (45%)	16 (57%)	4 (22%)	0.05
	No	16 (55%)	12(43%)	14(78%)	
Salt intake	High	5 (17%)	11 (39%)	7 (39%)	0.25
	Low	19 (66%)	16 (57%)	10 (56%)	
Age		56±12	54±13	54±12	0.76
BMI		29±6	28±6	27±4	0.60
Magnesium		185±74	199±60	186±67	0.70
Calcium		161±68	174±54	161±61	0.69
Potassium		3314±1457	3605±1162	3319±1297	0.66

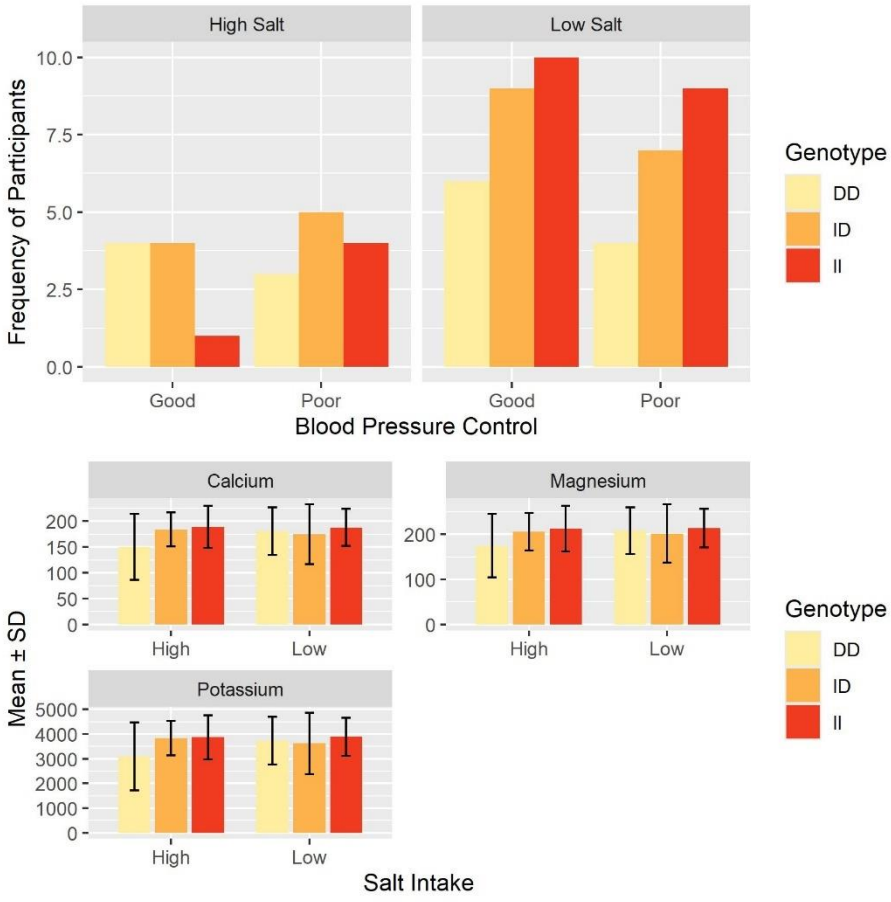

Fig. 2: Diet-gene interaction of ACE I/D polymorphism and Salt intake management in hypertensive patients. Number of individuals complying with blood pressure control management in conditions of high salt and low salt intake. On the other hand, Salt intake (Calcium, Magnesium, Potassium) and genotypes.

Discussion

Demographic variables such as gender, body weight, smoking habits, and family history of hypertension could play role in hypertension. In this study, gender distribution exhibited a significant difference (p=0.004) which is in agreement with previous studies (Bayorh *et al*, 2001; He *et al*, 2009). This gender difference in hypertension prevalence could be different in different studies due to variation in population or sample size (Patel *et al*, 2022). Additionally, overweight and obesity influence the overall risk of developing hypertension (Tang *et al*, 2022). In current study, the hypertensive group revealed notably elevated BMI (28±6) in contrast to the normotensive group (24±4). This finding is supported by the association between higher BMI and hypertension in previous studies (Hussain *et al*, 2018). Furthermore, smoking is likely attributed to an accelerated atherosclerosis process and hypertension (Virdis *et al*, 2010). There was a notable increase in the occurrence of smoking among hypertensive individuals in contrast to those with normal blood pressure.

Dietary factors, including salt intake, magnesium, calcium, and potassium levels, did not show significant differences in accordance with ACE I/D genotypes in hypertensive patients. This indicated that ACE I/D polymorphism may not directly influence these dietary factors in the study population. These results are in agreement with the previous studies that determine no association of ACE I/D polymorphism with CRF, diet, and physical activity (Montes-de-Oca-García *et al*, 2021). Reducing daily salt consumption by 2.3 grams within the context of the DASH diet results in less than half the impact on systolic blood pressure (SBP) and diastolic blood pressure (DBP) compared to not adhering to the DASH diet (-3.0/-1.6 mmHg versus -6.7/-3.5 mmHg, respectively).(Filippou *et al*, 2022). While their effects are not entirely cumulative, the combined implementation of both reduced sodium intake and the DASH diet yields a more substantial reduction in SBP and DBP (-8.9/-4.5 mmHg) compared to either dietary approach in isolation. Notably, various dietary elements, including salt intake, magnesium, calcium, and potassium levels, exhibit noteworthy associations with hypertension (Karppanen and Mervaala, 2006). The hypertensive group displayed a higher proportion of individuals with elevated salt intake (n=25) when contrasted with the normotensive group (n=5) ($p<0.001$). Furthermore, magnesium and calcium levels were notably lower among hypertensive individuals in comparison to their normotensive counterparts ($p<0.001$ and p=0.003, respectively). Distinct differences in potassium levels were also observed between the two groups (p=0.008).

Many researches had been publishing about role of ACE gene in hypertension. ACE gene has shown multiple phenotypes that could be associated with hypertension. For instance, ACE ID polymorphism was found associated with hypertension in Chinese population (Nawaz and Hasnain, 2011). In current study, characterization of anthropometric, clinical, and dietary intake revealed significant differences between normotensive and hypertensive individuals for various parameters. Notably, hypertensive individuals were found to be older (mean age 55±13) compared to normotensive individuals (mean age 38±8) with a highly significant p-value of less than 0.001. This age difference was consistent with hypertension being more prevalent in older populations.

Previously published data showed the occurrence of the I allele within the ACE I/D polymorphism shows a significant association with salt-sensitive hypertension. Moreover, the reaction of blood pressure to increased salt consumption varies among different genotypes of ACE I/D and 11betaHSD G534A, indicating that these polymorphisms could potentially serve as valuable genetic markers for identifying salt sensitivity.(Poch *et al*, 2001) This research showed the interaction between ACE I/D polymorphism and salt intake on anthropometric, clinical, and dietary factors in hypertensive patients (Razaq *et al*, 2023). The results suggest that the interaction of ACE I/D polymorphism and salt intake does not significantly influence BP control, magnesium, calcium, and potassium levels in hypertensive patients. The prevalence of hypertension did not differ significantly based on ACE I/D polymorphism. The distribution of the three genotypes (II, ID, DD) was insignificant between individuals with suboptimal BP control and the individuals with effective BP control. In previous studies, such variations have already been determined when these genotypes were compared (Birhan *et al*, 2022).

In conclusion, this study highlighted valuable insights into the characterization of anthropometric, clinical, and dietary variables in individuals with hypertension. Furthermore, it also explains the interaction of the factors with ACE I/D polymorphism and salt consumption. The findings showed the connections among age, BMI, blood pressure, smoking, family history, and hypertension. However, the study could not strongly support significant interactions between ACE I/D polymorphism and dietary factors in hypertensive patients. It implies that other genetic and environmental factors could be influencing the onset and management of hypertension. To validate and build upon these conclusions, further research with larger participant pools and more diverse populations is necessary

Declaration of Competing Interest

The authors declare that they have no competing or conflict of interests.

Author Contributions

FN: Conceptualization, Methodology, formal analysis, Writing—original draft preparation. **HS:** Conceptualization, Methodology, formal analysis, **QAH:** Formal analysis, Writing—review and editing. **MH:** Conceptualization, formal analysis, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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