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Research Article

Association Between Cytochrome P450 2C9 Gene Polymorphisms and Blood Pressure in Response to Losartan

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Abstract

To identify the genetic contribution to the variation in blood pressure (BP) response to angiotensin receptor blockers (ARBs), single-nucleotide polymorphisms (SNPs) in the cytochrome P450 (CYP) 2C9 gene were evaluated for their association with BP response to Losartan in Pakistani patients with hypertension. We selected 100 hypertensive patients and genotyped them for *CYP2C9**1, *2, and *3. Our results show that 40% of patients were responsive to antihypertensive treatment as per the defined criteria (a reduction of at least 10mm systolic & 5mm diastolic). More than 63% of patients who had wild type *CYP2C9* genotype did not respond to antihypertensive therapy; the rest did respond to the antihypertensive therapy. Similarly, more than 46% of patients having one of the polymorphic genotypes did not respond to therapy, while the rest did. The difference between the two groups, however, was not statistically significantly different. Hence polymorphism in the *CYP2C9* gene in our hypertensive cohort had no association with the antihypertensive therapy, including Losartan. Since our study has a relatively low sample size, we encourage larger studies to replicate and confirm these findings.

Keywords: Losartan, hypertension, *CYP2C9* polymorphisms, antihypertensive

Introduction

Hypertension is one of the leading causes of cardiovascular morbidity and mortality. A 15% and 40% decrease in the incidence of myocardial infarction and ischemic stroke has been reported with controlling BP(Collins et al. 1990). Hypertension has a complex etiology, with multiple players controlling various aspects of blood, exerting pressure against the vasculature (Figure 1). One of the first-line agents for hypertensive regulation that target the renin-angiotensin-aldosterone system (RAAS) is angiotensin receptor blockers, commonly known as ARBs(Koopmans, Insel, and Michel 2003). These classes of drugs reduce blood pressure by ensuring angiotensin II (AngII) does not bind to its specific molecular target, which is the angiotensin II receptor (Figure 2) (Frampton and Peters 1995; Oates, Wood, and Williams 1988). However, the ARB effect is greatly influenced by inter-individual and ethnic population variations (Kaplan, Sproul, and Mulcahy 1993; Mr et al. 1995). Therefore, it can be stated that genotypes may have a vital contribution in the antihypertensive response invoked by ARBs.

For quite some time, Losartan has been used to reduce the risk of cardiovascular disease by lowering blood pressure through the selective blockade of the AngII receptor. Losartan is administered as a prodrug, and for the drug to induce its antihypertensive effect, conversion into its active metabolite by the CYP2C9 hepatic enzyme is a pre-requisite (Babaoglu et al. 2004). Various

investigations have concluded the antihypertensive effect is decreased in patients possessing the CYP2C9*3 allele due to reduced oxidation of Losartan (Babaoglu et al. 2004; Sekino et al. 2003). CYP2C9, however, inactivates irbesartan, another ARB. Hong et al, 2005 reported that plasma irbesartan levels were considerably higher in patients with the CYP2C9*3 allele (X et al. 2005). Furthermore, 6 hours post-administration, irbesartan levels remain elevated in hypertensive Chinese patients with the CYP2C9*3 allele (Chen et al. 2006; St et al. 2001). Henceforth, it can be discerned that the CYP2C9*3 allele is a major player in determining the individualistic response to BP-lowering pharmacological agents. Polymorphisms in the gene for the CYP2C9 enzyme have been known to inculcate variations in the antihypertensive response to ARBs. Extensive polymorphisms at the genetic level have been observed in different races, nationalities, and geographical profiles. Our investigation aims to delineate the relationship between the prevalence of the different CYP2C9 genotypes: rs1057910, A1075C, Ile359Leu, CYP2C9*3 and blood pressure of hypertensive Pakistani population that are on Losartan, an angiotensin receptor antagonist. The study outcome may serve to give vital information on how to select and use ARBs, mainly Losartan.

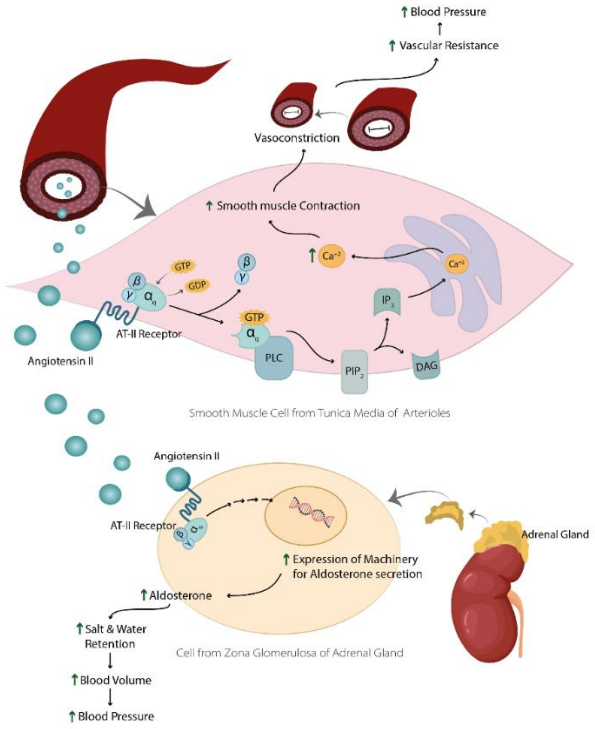

Figure 1: Various factors affecting hypertension

Methods & Materials

Patients

Between the ages of 27 and 75, 100 moderately hypertensive patients comprised of our patient cohort. Patients with consistent diastolic blood pressure of 90mmHg even after the use of antihypertensive medication or in repeated measurements were chosen to be part of our study. Secondary hypertension, extensive comorbidity, and/or the use of three or more antihypertensive agents made up the exclusion criteria. 97 out of 100 patients were already taken BP lowering drugs prior to the study.

50mg Losartan for 14 days was given to each patient. A 2-week placebo period was conducted prior to administration of the drug. BP of all 100 patients taking Losartan was measured. SBP 145mmHg and DBP 98mmHG was recorded by the end of the placebo duration. Baseline lab work was conducted. The study was conducted in accordance with the Ethical guidelines of Shifa International Hospitals and all patients provided written informed consent to the research.

Genotype Analysis

Standard procedures for genomic DNA extraction were performed where blood was drawn from the peripheral vein. As per already published studies three gene polymorphs were investigated. The DNA section of interest was identified by PCR amplification, after which sequencing from a commercial sequencing facility in China.

Statistical Analysis

The version 26.0 of the IBM Statistical Package for Social Sciences SPSS was employed for data-keeping and analysis. Mean ± SD was used to represent continuous variables whereas discrete variables were represented as percentage and frequencies. Losartan response served as the parameter to classify the patients; responsive participants showed a 5mmHg and 10mmHg decrease in diastolic and systolic blood pressures respectively. Chi-square test was used to compare proportion of responsive subjects in genotype groups. P-value of 0.05 or less was regarded as statistically significant.

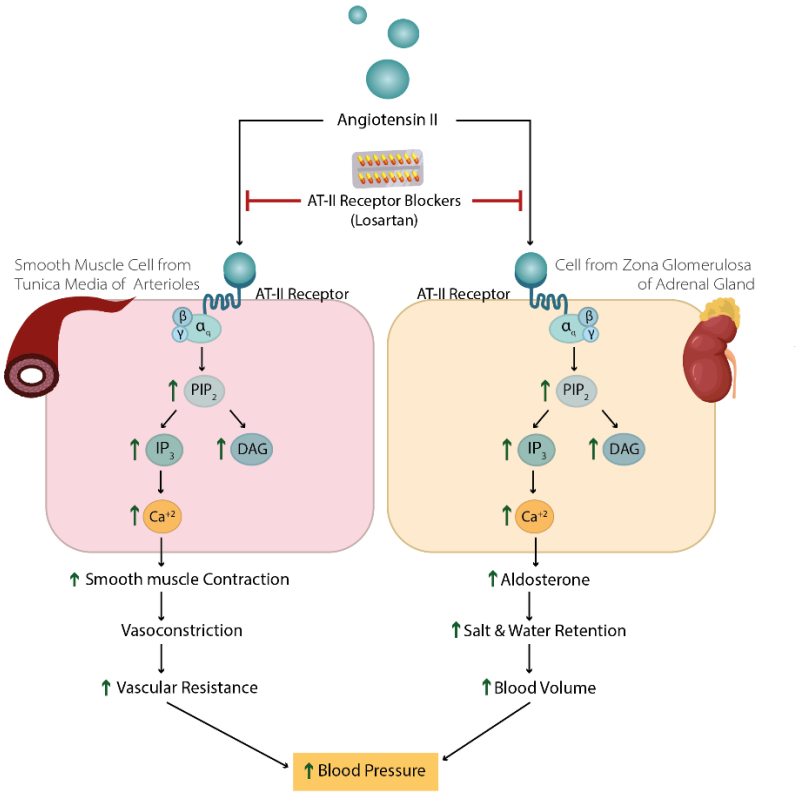

Figure 2: Mechanism of action of ARBs.

Table 1: Baseline and Demographic characteristics of the investigated population.

Characteristics	Values
Age (years), mean ±SD	48±8
Females, n (%)	67 (67)
Smokers, n (%)	8 (8)
SBP (mmHg)	145 ±13
DBP (mmHg)	98 ±12
Co-morbid Conditions	
Arthritis	18 (18)
Diabetes	3 (3)
Concurrent Medications	
Aspirin	99 (99)
Atenolol	100 (100)
Amlodipine	2 (2)
Glyceryl Trinitrate	1 (1)
Omeprazole	1 (1)

Results

Table 1 outlines demographic features and baseline parameters of the hypertensive Pakistani population investigated of this research. 48±12 years was the mean patient age where 67% participants being female. Smokers comprised only 8% of the cohort. The mean BP at the start of Losartan treatment was 145 ±13 (systolic) and 98 ±12 (diastolic). The co-morbidities that were most commonly seen were arthritis (18%) and diabetes (3%). Concurrent medications used by the cohort include aspirin (99%), atenolol (100%), amlodipine (2%), glyceryl trinitrate (1), and omeprazole (1%). Hematological parameters of our hypertensive cohort are depicted in the table 2. Mean values of RBC, Hb, HCT, platelets, MCV, MCH, MCHC, WBC, % neutrophils, % lymphocytes, and uric acid levels in the blood along with standard deviations are shown and were within the normal range for these parameters.

Table 2: Hematological parameters of the investigated population.

Variable	Mean	Standard Deviation
WBC [10^3/ul]	8.600	2.2182
RBC [10^6/ul]	5.386	0.6020
Hb [g/dl]	11.907	1.9087
HCT [%]	42.757	3.7709
MCV [fL]	80.563	5.5284
MCH [Pg]	24.797	2.3394
MCHC [g/dl]	30.690	3.0863
Platelets [10^3/ul]	265.39	60.153
Neutrophils [%]	64.00	8.415
Lymphocytes [%]	32.14	7.506
Uric Acid [mg/dl]	4.909	0.7552

The distribution of the CYP2C9 genotypes in the investigated cohort is shown in the table 3. A majority of the participants had wild type genotype (*1*1). Polymorphic genotypes accounted for 21% of the cohort. However, no *3*3 genotype was observed in the hypertensive Pakistani population investigated in this study. The most prevalent of the polymorphic genotypes was *1*3 (8%), followed by *1*2 (7%), *2*3 (5%), and *2*2 (1%).

Table 3: Distribution of CYP2C9 genotypes in the investigated hypertensive population.

Genotype	N	Observed genotype frequency (CI)	Expected genotype frequency by HW law
*1*1	79	79 (75.3-84.4)	78.6
*1*2	7	7 (5.1-9.2)	7.7
*1*3	8	8 (5.3-9.6)	7.9
*2*2	1	1 (0.5-2.6)	0.6
*2*3	5	5 (3.3-7.9)	5.2

Table 4 shows that 40% patients were responsive to antihypertensive treatment as per the defined criteria (a reduction of at least 10mm systolic & 5mm diastolic). More than 63% patients who had wild type *CYP2C9* genotype did not respond to antihypertensive therapy, the rest did respond to the antihypertensive therapy. Similarly, more than 46% patients having one of the polymorphic genotypes did not respond to therapy while the rest did. The results between both the groups were not statically significant and hence polymorphism in *CYP2C9* gene in our hypertensive cohort had no association with the antihypertensive therapy including Losartan.

Table 4: Chi-squared analysis of antihypertensive treatment response and polymorphisms in *CYP2C9* gene.

Genotype	Treatment resistant	Treatment responsive	Patients	
Wild type	50	29	79	
	63.3%	36.7%	100.0%	
Polymorphic	10	11	21	
	47.6%	52.4%	100.0%	p-value: 0.193

Discussion

The patients undergoing the two-week Losartan regimen, in our investigation, showed no significant association in antihypertensive response and CYP2C9 genetic polymorphism. There have been previous reports of SNPs association in different genes with response to blood pressure to certain blood pressuring reducing drugs. Within angiotensin gene (*AGT*), The SNP rs699, has been associated with lowered BP in response to commonly termed "pril" which are in essence Angiotensin Converting Enzyme Inhibitors treatment in one study(Hingorani et al. 1995), which has not been the case in other researches(C et al. 1996; Uf et al. 1998). In the pre-

sent study, 2 *CYP2C9* SNPs exhibited no association with Losartan BP response. however, prevalence of minor alleles of these SNPs in this Pakistani hypertensive cohort were similar to those of healthy human population reported earlier(Ahmed et al. 2020). In another study a separate the rs7079 SNP in *AGT* gene and reduced blood pressure due to ACEi did exhibit an association: Inside the 3′-untranslated region of the *AGT* gene, a C-to-A polymorphism is detected in this SNP. Via the Transcription Element Search System, (TESS v4.0; http://www.cbil.upenn.edu/tess), it was seen that in the presence of C allele sequence a ubiquitous factor AP4–binding site was present. The A allele sequence demonstrated a loss in the AP4-binding site. Subsequently, the possible purpose of the SNP could have a relationship with a likely binding site for the AP4 transcription factor. An SNP in the angiotensin receptor 1 gene (*AGTR1* SNP A-1166C (rs5186)) was associated with SBP response to diuretic therapy in black women (Frazier et al. 2004).

Auxiliary factors not being controlled was the biggest limitation of this study, as it was a post-marketing investigation rather than a randomized clinical trial. For instance, in 100 patients studied, a large proportion of them were on drug treatment prior to becoming part of the investigation, and their BP was not controlled at an ideal level, with the end goal that the mean baseline BP in this cohort was 145/98 mmHg. Another limitation is the small size of our study. Extensive studies with more patients are necessary to validate these results.

In summary, it is expected that the absence of *CYP2C9* associations observed here will not deter further, larger studies to confirm or refute the finding of the current study. However, at least in the Pakistani hypertensive cohort investigated in this study, no such association was found.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

There was no specific funding available for this project.

Study Approval

Yes. The study was approved by the Institutional Review Board & Ethics Committee of the Bahaud Din Zakariya University Multan

Consent Forms

Consent forms were signed by the patients, and are available with the authors.

Authors Contribution

II conceptualized the study, AQ, WA, and SJ collected samples, carried out the experimental work, helped with analysis and writing the first draft, II and IK supervised the whole project and wrote the final manuscript.

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