

**Research Article****Efficacy of the Angiotensin-Converting Enzyme Inhibitors in a Pakistani Hypertensive Cohort and its Associations with Genetic Variation in *SCLO1B1* Gene**Fawad Ali^{1*}, Taseer Ahmad²¹Department of Pharmacy, Kohat University of Science & Technology, Kohat, Pakistan.²College of Pharmacy, University of Sargodha, Sargodha, Pakistan.

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Abstract

A significant proportion of individuals at high risk of cardiovascular risks are prescribed Angiotensin-converting enzyme inhibitors (ACEIs). This study investigated the potential link between *SLCO1B1* genetic variations and the effectiveness of ACEIs in managing hypertension within a Pakistani cohort. Designed as a prospective cohort study, it was conducted at a tertiary care hospital, enrolling 83 patients through a convenient sampling approach. After informed consent, 2 mL of blood was withdrawn from each participant. After the DNA extraction from blood samples, single nucleotide polymorphisms (SNPs) in the *SLCO1B1* gene were determined through partial sequencing. Furthermore, a structured questionnaire was employed to gather data. The findings indicated a notable reduction in both systolic blood pressure (SBP) and diastolic blood pressure (DBP) following two weeks of ACEI therapy. However, no significant correlation was detected between ACEI efficacy and the two SNPs examined in this population. Expanding this research to larger and more genetically diverse cohorts could provide a clearer understanding of the association between genetic variants and ACEI therapeutic response.

Keywords: Angiotensin-converting enzyme, hypertension, genetic polymorphism, *SCLO1B1*, therapeutic response**1. Introduction**

Hypertension is a significant risk factor for conditions like myocardial infarction (MI), stroke, and critical renal diseases (James et al. 2014). Within the past three decades, the prevalence has grown to twice its size. In 1990, 650 million people were suffering from high blood pressure, and in 2019, that number stood at 1.3 billion (WHO 2023). In Pakistan, the prevalence is notably high in individuals older than 15 years, impacting 16% of the rural population, and from urban areas, 21.6% of the population is affected. Most hypertensive patients in Pakistan remain undiagnosed. Approximately 5.3 million women and 5.5 million men are affected by hypertension in the

country, yet fewer than 3% receive proper treatment to control their blood pressure (Kearney et al. 2005, Soomro, Abro, and Shah 2013, Ahmed et al. 2008). Moreover, of those over the age of 45, 1/3rd of Pakistanis suffer from elevated blood pressure (Ghaffar, Reddy, and Singhi 2004).

Various synthetic and natural drugs have been on the market for blood pressure management (Ali, Shahid, and Shahid 2023, Hussain et al. 2010). Joint National Committee VII (JNC-7) ranks Angiotensin-converting enzyme inhibitors (ACEIs) among the safest antihypertensive agents for patients with comorbidities such as diabetes, as the vital organs like the kidneys and the heart largely

remain unimpacted by ACEIs (Khalil et al. 2001). The safety profile is the reason behind the widespread prescription of ACEIs for treating hypertension. With around 30 million consumers in the world, the size of the ACEIs market is valued at 3,456 million USD globally (Messerli et al. 2018). ACEIs are frequently prescribed in Pakistan to patients hospitalized for MI treatment (Fiaz Alam 2024).

The solute carrier organic anion transporter family member 1B1 (SLCO1B1) is encoded by the *SLCO1B1* gene. SLCO1B1 or OATP1B1 is important for the hepatic clearance of numerous pharmacological agents, including ACEIs (Pasanen et al. 2007). This gene's function drug uptake from the blood into the liver cells makes their genetic expression vital to ACEIs' therapeutic outcome and potential adverse effects. The *SLCO1B1* gene is found to be highly polymorphic with > 190 single nucleotide polymorphisms (SNPs) (Liu et al. 2006). Among these, 521T>C (rs4149056) and 597C>T (rs2291075), the most frequent SNPs, are linked to the pharmacokinetics as well as pharmacodynamics variations of SLCO1B1 substrates (Oshiro et al. 2010). Notably, the association between *SLCO1B1* SNPs and the ACEIs' antihypertensive efficacy remains unexplored in Pakistan. Given the widespread use of ACEIs, healthcare providers must understand individual responses to these agents. Therefore, the objective of this study is to explore whether the efficacy of ACEIs in a cohort of hypertensive patients in Pakistan is influenced by genetic variations, specifically SNPs in the *SLCO1B1* gene.

2. Materials and Methods

Through convenient sampling, ambulatory patients visiting a Pakistani tertiary care hospital were recruited in this prospective cohort study. The hospital's institutional review board (IRB) and ethics committee (EC) assented to the study. After giving WHO-mandated informed consent, participants complete a structured

questionnaire. Additionally, baseline data collected from participants included gender, height, body weight, age, smoking status, etc. Information on ACEI use, such as the active compound and duration of therapy, was noted. Only patients fulfilling the inclusion criteria participated in the study. Patients suffering from any respiratory condition were excluded. Eligible participants using ACEIs were interviewed on a fortnight after therapy through a phone call.

Each participant provided venous blood (2 mL) in a sterile ethylenediaminetetraacetic acid (EDTA) tube. Genomic DNA from leukocytes was extracted following a previously established protocol and stored at -20°C (Hussain et al. 2023). DNA concentration was measured using a NanoDrop spectrophotometer. Afterwards, the *SLCO1B1* gene was subjected to targeted sequencing using SeqStudio™ (Applied Biosystems, USA), where the focus was *SLCO1B1*'s hypervariable region. This approach has previously provided reliable outcomes (Ahmed et al. 2022). Cycle-sequencing of these amplified PCR products was done by using a BDT v.3.1 master mix, the manufacturer's instructions were followed for both forward and reverse primers. To purify the cycle-sequenced products under the manufacturer's protocol, the BigDye Xterminator™ (ThermoFisher Scientific, USA) kit was used. For analysis, samples were loaded and run using parameters based on the kits. For electrophoresis, a medium run was selected.

After sequencing, the DNA sequence reads were assessed for quality using the Staden Package (Staden, Beal, and Bonfield 1999) and Finch TV v1.4.0

(<http://www.geospiza.com/Products/finchtv.shtml>) to ensure accuracy. High-quality sequence reads were assembled using Lasergene v.7.1 (DNASTAR Inc., USA) and Bio-Edit software (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) following the methodology described by Khokhar et al. (Khokhar et al. 2021).

Table 1: Descriptive characteristics of the study population. BMI = body mass index, ACEIs = Angiotensin-converting enzyme inhibitors.

Variable	Category	Frequency/Mean ± SD
Sex	Male n (%)	41 (49.4)
	Female n (%)	42 (50.6)
Age (Years)		50.83±10.91
BMI (Kg/m ²)		24.59±7.10
Ethnicity	Kashmiri n (%)	5 (6.0)
	Pathan n (%)	18 (21.7)
	Punjabi n (%)	58 (69.9)
	Sindhi n (%)	2 (2.4)
Family History Of CVD	No n (%)	36 (43.4)
	Yes n (%)	47 (56.6)
Smoking	No n (%)	64 (77.1)
	Yes n (%)	19 (22.9)
ACEI Types	Captopril n (%)	16 (19.3)
	Enalapril n (%)	9 (10.8)
	Lisinopril n (%)	6 (7.2)
	Ramipril n (%)	52 (62.7)

The high-quality filtered sequence data was analyzed against the GRCh38.p14 reference genome from the Ensemble database using Blast (Howe et al. 2021). To ensure accurate alignment, sequences from the entire cohort were processed using the MUSCLE algorithm (Edgar 2004) in MEGA-X (Kumar et al. 2018). The designated in-house alignment region for the SLCO1B1 gene (12:21178627–21179056) was successfully analyzed in the Ensemble database, encompassing partial exon 6, the complete exon 7, the entirety of intron 7, and a segment of intron 8. Haplotypes were identified through DnaSP v6.12 software (Rozas et al. 2017). The output from DnaSP v6.12 was converted into the Arlequin project file format (.arp), which was then used to determine the number of polymorphic sites and haplotype frequencies. (Excoffier, Laval, and Schneider 2005). In Arlequin v3.5, a new project was configured, categorizing all samples (e.g., coughers vs. non-coughers) and specifying the molecular data type (DNA sequences). Both haplotypic and locus-level parameters were adjusted to detect

shared haplotypes across samples. The software ultimately generated a comprehensive haplotype list along with frequency distributions.

This study was analyzed through descriptive and inferential statistics. Descriptive statistics, including frequency, standard deviation, percentages, and mean, were calculated for demographic and clinical variables. Paired t-tests were performed to compare systolic blood pressure (SBP) and diastolic blood pressure (DBP) on the 1st day versus the 14th day. An independent sample t-test was selected to compare SBP-DBP differences (day 1 and day 14) between different genotypes (CC and CT) of rs2291075. Analysis of variance (ANOVA) helped assess differences in SBP and DBP (day 1 and day 14) among different genotypes (CC, CT, and TT) of rs4149057, as well as for different genotype combinations (CC-CT, CT-CC, CT-CT, CC-TT, TT-CC, and TT-CT) of rs4149057 and rs2291075. The statistical analysis was performed using SPSS software.

Table 2: Frequencies and percentages for SNP with their genotype.

SNP	Genotypes	Frequency (%)
rs4149057	CC	2 (2.4)
	CT	21 (25.3)
	TT	60 (72.3)
rs2291075	CC	50 (60.2)
	CT	33 (39.8)

3. Results

The investigation of basic demographic and clinical parameters of the studied population revealed that there were 41 male participants (49.4%), and 42 female patients (50.6%) (Table 1). The mean and standard deviation for age and BMI were 50.83±10.91 years and 24.59±7.10 kg/m², respectively. There were 5(6.0%) Kashmiris, 18(21.7%) were Pathan, 58(69.9%) were Punjabi and 2(2.4%) of the patients were Sindhi. About 36(43.4%) of the patients had no family history of CVD, while 47(56.6%) had a family history of CVD. Also, 64(77.1%) of the patients were non-smokers, while 19(22.9%) of the patients were smokers. As for different types of ACEIs, 16(19.3%) used captopril, 9(10.8%) used enalapril, 6(7.2%) were taking lisinopril and 52(62.7%) used ramipril.

We found two SNPs (rs4149507 and rs2291075) during our investigation of the partial SCO1B1 gene sequencing. Table 2 describes the frequencies and percentages for these genotypes with their categories. For the SNP rs4149507, there were 2(2.4%) respondents who had CC, 21(25.3%) respondents had CT, and 60(72.3%) of the respondents had TT category. For the SNP rs2291075, there were 50(60.2%) respondents with CC and 33(39.8%) of the respondents with CT genotypes. No TT genotype was found for rs2291075.

Table 3 shows the types and frequencies of concomitant medicines used by patients. 29 patients were using ARBs, NSAIDs were being used by 27 patients, β -blockers by 18 patients, statins by 14 patients, diuretics by 10 patients, biguanides by 6 patients, and CCB by 5 patients. Similarly, 3 patients were using plasminogen

activators, 3 were using proton-pump inhibitors, 2 patients were using chemotherapies, and 2 were using anticholinergic. Furthermore, there was one patient who was using anticonvulsants (sodium channel blockers), 1 patient was using cholinesterase inhibitors, and 1 was using an opioid receptor agonist.

Table 4 reveals the findings of the paired t-test for mean comparisons of SBP and DBP (between day 1 and day 14) among the patients. The results indicated significant mean differences in SBP with $t(82)=7.256$ and $p<.001$. These results show that the mean values of SBP for day 1 (mean = 131.81, SD = 8.06) subsequently decreased on day 14 (mean=86.45, SD=7.84). The finding also indicated significant mean differences in DBP with $t(82)=7.821$ and $p<.001$. Results show that the mean score for DBP for day 1 (mean=86.45, SD=5.50) subsequently decreased on day 14 (mean=78.73, SD=6.43).

Table 5 represents the results for the difference between SBP and DBP (for day 1 and day 14) between different genotypes (CC and CT) of rs2291075. From the results, we conclude that the difference between SBP and DBP (for day 1 and day 14) was not significantly different between different genotypes (CC and CT) of rs2291075 with p-values of 0.602 and 0.794, respectively.

Table 6 shows the results of ANOVA for the difference between SBP and DBP (for day 1 and day 14) among different genotypes (CC, CT, and TT) of rs4149057. From the results, it was obvious that the difference between SBP and DBP (for day 1 and day 14) did not significantly differ among different genotypes (CC, CT, and

Table 3: Frequencies for different type of medicine used by patients.

Sr. No.	Type of Medicine	No. of Patients Use
1	ARB	29
2	NSAIDs	27
3	β	18
4	Statins	14
5	Diuretic	10
6	Biguanides	6
7	CCB	5
8	Plasminogen activator	3
9	Proton-pump Inhibitors	3
10	Chemotherapies	2
11	Anticholinergic	2
12	Anticonvulsants (Sodium Chanel Blocker)	1
13	Cholinesterase Inhibitors	1
14	Opioid Receptor Agonist	1

Table 4: Results of pared t-test for mean comparisons of SBP and DBP (day 1 and day 14).

Variables	Day 1		Day 14		t(82)	p-value	95% CI	
	Mean	SD	Mean	SD			LL	UL
SBP	131.81	8.06	124.16	7.84	7.256	.000	5.55	9.75
DBP	86.45	5.50	78.73	6.43	7.821	.000	5.75	9.67

***p < .001.

TT) of rs4149057 with p-values 0.235 and 0.123, respectively.

Table 7 shows the results of ANOVA for the difference between SBP and DBP (for day 1 and day 14) among and across different categories of genotypes (CC-CT, CT- CC, CT-CT, CC-TT, TT-CC, and TT-CT) of rs4149057 and rs2291075. From this investigation, we conclude that differences between SBP and DBP (for day 1 and day 14) did not significantly differ among different cross categories of genotypes (CC-CT, CT- CC, CT-CT, CC-TT, TT-CC, and TT-CT) of rs4149057 and rs2291075 with p-values 0.217 and 0.177, respectively.

4. Discussion

The study found that the frequencies of ACEI usage were ramipril (52), captopril (16), enalapril (9), and lisinopril (6). ACEIs used in this investigation were efficient in decreasing

both the SBP and DBP. However, the current study found that neither of the SNPs (rs4149507 and rs2291075) was associated with the therapeutic efficacy of the ACEIs in reducing SBP and DBP.

This study demonstrates that ACEIs significantly reduce both SBP and DBP two weeks after the baseline measurements. On average, SBP fell by 7.84 mm, whereas DBP decreased by 6.43 mm after two weeks of ACEI treatment compared to baseline values. However, the study could not establish a strong association between SNPs and the efficacy of ACEIs. Previous research has shown that *SLCO1B1* variants were linked with toxicity, adverse reactions, or the therapeutic efficacy of OATP1B1-transported drugs, such as toxicities induced by irinotecan, statin-induced muscle pain, and elimination of methotrexate (Carr et al. 2013, Group 2008, Hussain et al. 2023). Research

Table 5: The comparison of difference between SBP and DBP (for day 1 and day 14) between different genotypes (CC and CT) of rs2291075

Variables	<i>M ± SD</i>		t	p	95% CI	
	CC (n=50)	CT (n=33)			LL	UL
SBP	7.20±9.49	8.33±9.90	-.524	.602	-5.439	3.173
DBP	7.50±8.82	8.03±9.35	-.262	.794	-4.625	3.501

Table 6. The comparison of difference between SBP and DBP (for day 1 and day 14) among different genotypes (CC, CT and TT) of rs4149057

ANOVA Table						
Variable s	SOV	Sum of Squares	df	Mean Square	F	p
SBP	Between Groups	269.248	2	134.624	1.476	.23
	Within Groups	7297.619	80	91.220		
	Total	7566.867	82			
DBP	Between Groups	337.501	2	168.750	2.151	.12
	Within Groups	6277.560	80	78.469		
	Total	6615.060	82			

Table 7. The comparison of difference between SBP and DBP (for day 1 and day 14) among different cross categories of genotypes (CC-CT, CT-CC, CT-CT, CC-TT, TT-CC and TT-CT) of rs4149057 and rs2291075.

Variable s	SOV	Sum of Squares	df	Mean Square	F	p
SBP	Between Groups	533.485	4	133.371	1.479	.21
	Within Groups	7033.383	78	90.172		
	Total	7566.867	82			
DBP	Between Groups	508.612	4	127.153	1.624	.17
	Within Groups	6106.448	78	78.288		
	Total	6615.060	82			

has also linked *SLCO1B1* 521T > C polymorphism to differences in drug responses, while findings related to the 597C > T variant, which could increase, decrease, or have no effect on transport activity, remain inconclusive (Niemi, Pasanen, and Neuvonen 2011). In a Chinese study, the variant 521T > C was found to be a critical factor in ACEIs pharmacokinetics (Tian et al. 2011), while in Russians, *SLCO1B1* rs2306283 was associated with dry cough induced by enalapril (Sychev et al. 2023).

Another study stated that the *SLCO1B1* 521T > C (Val174Ala) variant significantly alters the risk of cough in the Chinese population induced by enalapril (Luo et al. 2015). The lack of a significant association between ACEI efficacy and SNPs in our study may be attributed to genetic differences within our population or to the relatively smaller number of samples. Beyond population-specific genetic variations and the limited sample size, environmental factors—including diet, blood-oxygen levels, temperature, humidity, light cycles, and

exposure to mutagens—may also impact the potential link to ACEI-induced cough, potentially masking these associations.

The lack of a significant genetic association in our study contrasts with other research showing a strong correlation. Previous studies have suggested that genotyping *SLCO1B1* variants may be a useful strategy for improving the efficacy and safety of enalapril treatment in China (Luo et al. 2015). These findings emphasize the importance of pharmacogenetic markers for ACEI-based therapy optimization. However, broad-scale trials, including functional analysis investigation into the underlying molecular mechanisms, should be explored to clarify study findings.

Despite the validity and significance of the current study's findings, several limitations need to be acknowledged. Although partial gene sequencing provided reliable results in previous studies (Ahmed et al. 2022), it may have missed critical information that could be found through the entire gene sequencing. Additionally, the smaller sample numbers hamper the ability to generalize these results, which could explain the failure to establish SNP-ACEI efficacy dynamics.

Conflict of Interest

The authors declare that they have no competing interests.

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Study Approval

This study was approved by the ethical committee of Kohat University of Science & Technology, Kohat, Pakistan.

Consent Forms

Consent forms are available with the authors.

Data Availability

All the data related to this study are available with the authors.

Authors Contribution

Both the authors contributed equally to this study.

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