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Original Article

Distribution of SLC6A4 Gene Polymorphisms in a Multi-Ethnic Pakistani Cohort with Major Depressive Disorder

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ABSTRACT

Objective: To determine the distribution of *SLC6A4* gene polymorphisms (5-HTTLPR and rs25531) in a multi-ethnic Pakistani population with Major Depressive Disorders.

Study Design: Multi-center cross-sectional study.

Place and Duration of Study: Tertiary care hospitals in Quetta, Pakistan from Apr 2023 to Oct 2024.

Methodology: Four hundred thirty Pakistani patients with Major Depressive Disorder from five ethnic groups (Punjabi, Sindhi, Pathan, Baloch, Kashmiri) were enrolled. Genotyping for 5-HTTLPR and rs25531 polymorphisms was performed using allele-specific PCR followed by restriction fragment length polymorphism. Allele and genotype frequencies were calculated, Hardy-Weinberg equilibrium tested, and inter-ethnic comparisons made. Functional classification was based on tri-allelic system (LA, LG, S).

Results: A total of 430 participants were included in the study. Mean age was 32.29 ± 6.80 years. S allele was more common than the L allele, with frequencies of 494 (57.4%) and 366 (42.6%), respectively. Pathan group had the highest frequency of the L allele (47.3%) while Sindhi group had lowest (39.4%). LA allele was common in Pathans (36.5%) and least common in Sindhis (30.8%). Therefore, difference between in allele distribution and ethnic group was statistically significant as p value = 0.012 and differences in treatment response among the three genotype groups were also statistically significant as p value < 0.001.

Conclusion: Pakistani population shows unique *SLC6A4* genetic architecture with S allele predominance (0.574) and substantial LA allele representation (0.324). The high frequency of low-expression genotypes suggests many Pakistanis may be genetically predisposed to variable SSRI responses. These population-specific data are crucial for developing pharmacogenetic strategies in Pakistan.

Keywords: Gene polymorphisms, Pakistan, Major Depressive Disorder, SLC6A4 gene, Selective serotonin reuptake inhibitors.

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INTRODUCTION

Major Depressive Disorder (MDD) constitutes a significant public health burden in Pakistan, with prevalence estimates ranging from 10-16% of the adult population. The economic impact is substantial, with mental illness burden escalating alarmingly in recent years. Selective Serotonin Reuptake Inhibitors (SSRIs) like escitalopram remain first-line pharmacological treatments, yet response rates are variable, with only 40-60% of patients achieving remission with initial therapy. This treatment heterogeneity has spurred interest in pharmacogenetics, particularly the role of the serotonin transporter gene (SLC6A4), which encodes the primary target of SSRIs.[1,2]

The SLC6A4 gene contains two functionally significant polymorphisms: the serotonin transporter-linked polymorphic region (5-HTTLPR), a 44-base pair insertion/deletion resulting in long (L) and short (S) alleles[8] and rs25531, a single nucleotide polymorphism (A>G) within the L allele that creates functionally distinct sub-alleles (LA and LG).[3] The S and LG alleles are associated with reduced transcriptional efficiency and lower serotonin transporter expression compared to the LA allele. These functional differences translate to clinical outcomes, with meta-analyses indicating that L allele carriers, particularly LA homozygotes, show better response to SSRIs and fewer adverse effects (Figure-1).[4,5]

Allele frequencies of these polymorphisms show considerable ethnic variation globally.[6] European populations typically show L allele frequencies of 0.57-0.60, while East Asian populations show much lower L frequencies (0.20-0.30).[7,8] Pakistan, with its population of over 240 million comprising multiple ethnic groups (Punjabi, Sindhi, Pathan, Baloch, Kashmiri), represents a significant pharmacogenetic knowledge gap.

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Located at the crossroads of South Asia, Central Asia, and the Middle East, Pakistan's population has a complex genetic history with contributions from various ancestral populations, yet no comprehensive data exist on SLC6A4 polymorphism frequencies in the country.[9,10] This study aimed to establish the distribution of SLC6A4 polymorphisms in a multi-ethnic Pakistani cohort, analyze inter-ethnic variation, compare with global populations, and discuss implications for antidepressant pharmacogenetics in Pakistan.

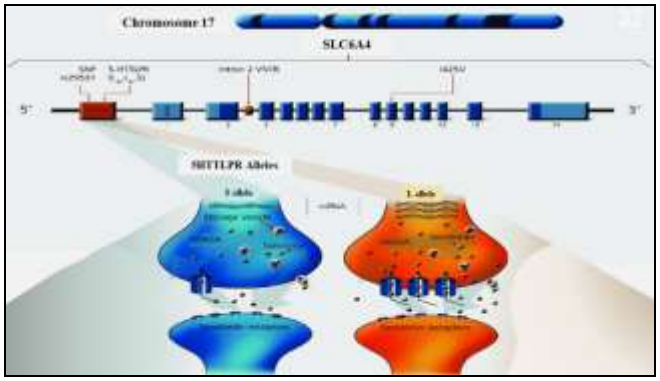

Figure-1: Polymorphic forms of SLC6A4 Gene

METHODOLOGY

This multi-center cross-sectional study was conducted after approval from the University of Haripur Institutional Review Board and local ethics committees of participating hospitals. Written informed consent was obtained from all participants.

Four hundred thirty Pakistani patients diagnosed with Major Depressive Disorder (MDD) according to DSM-IV criteria were enrolled from tertiary care hospitals across different centers between April 2023 and October 2024. Depression severity was assessed using the Hamilton Depression Rating Scale (HAMD-17). HAMD-17 scores of the participants were recorded at the start of the treatment and then at 4th, 8th and 12th weeks of therapy.

Inclusion Criteria: Patients aged 18-65 years freshly diagnosed patients of MDD who were started with Antidepressant therapy with Tab Esctalopram were included.

Exclusion Criteria: All those having history of any other illness were excluded from the study.

Ethnicity was self-reported and verified through parental birthplace. The sample represented all five major Pakistani ethnic groups: Punjabi, Sindhi, Pathan, Baloch, and Kashmiri/Northern Areas. Five milliliters of peripheral blood were collected in EDTA tubes from each participant. Genomic DNA was extracted using the EasyPure Genomic DNA Kit (TransGen Biotech, China) following manufacturer's protocol. A two-step genotyping approach was employed. First, 5-HTTLPR genotyping was performed using allele-specific PCR with primers: Forward 5'-GGCGTTGCCGCTCTGAATGC-3', Reverse 5'-GAGGGACTGAGCTGGACAACCAC-3'. PCR conditions: initial denaturation at 95°C for 5 minutes; 30 cycles of denaturation at 95°C for 55 seconds, annealing at 59°C for 50 seconds, extension at 72°C for 60 seconds; final extension at 72°C for 10 minutes. Second, rs25531 genotyping was performed by digesting PCR products with MspI restriction enzyme (New England Biolabs) at 37°C for 16 hours. Products were analyzed by 2% agarose gel electrophoresis. Fragment sizes: L allele=529 bp, S allele=484 bp; LA allele remains 529 bp after digestion, LG allele cuts to 340 bp + 189 bp (Figure-2).[11,12]

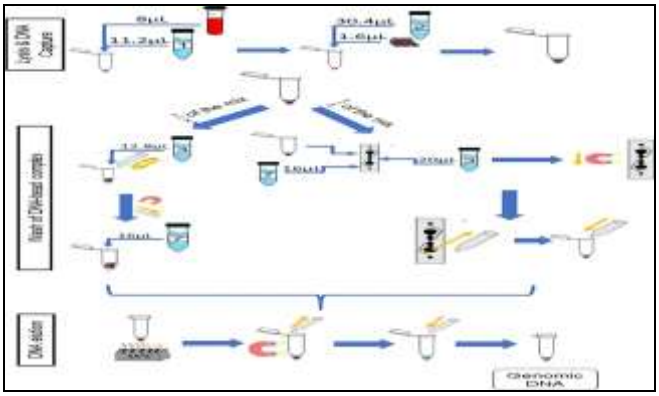

Figure-2: Process of DNA Extraction

Three classification systems were applied: biallelic (LL, LS, SS based on 5-HTTLPR), tri-allelic (LA/LA, LA/LG, LA/S, LG/LG, LG/S, S/S incorporating rs25531), and functional groups based on number of La alleles: 2L (LA/LA), 1L (LA/LG or LA/S), 0L (LG/LG, LG/S, or S/S).[13,14] Quality control measures included blinded genotyping, 10% random duplicates, and positive/negative controls in each run. Genotyping success rate was 99.5%.

Data were analyzed using Statistical Package for Social Sciences SPSS version 26 Continuous variables were tested for normality using the shapiro wilk test and presented as Mean±SD, while categorical variables were presented as frequency and Percentages. Baseline demographic and clinical characteristics were compared among genotype

groups using the chi square test for for categorical variables and repeated measures ANOVA for continuous variables. Changes in HAMD scores among the SLC6A4 genotype groups. The *p*-value≤0.05 was considered as significant.

RESULTS

A total of 430 participants were included in the study. Mean age was 32.29±6.80 years. 210(48.8%) participants were male while 220(51.2 %) were females. Participants were enrolled from all six provinces of Pakistan with highest representation from Punjab (43.9%) followed by Sindh (23.0%). The majority of the study participants had primary (37.9%) and secondary (46.0%) education shown in Table-I. Sallele was more common than the L allele, with frequencies of 494(57.4%) and 366(42.6%), respectively. Among the L allele variants, the LA allele was present in 279(32.4%) and the LG allele in 87(10.2%). The LS genotype was the most common, observed in 202 (47.0%) participants, followed by the SS genotype in 146(33.9%) and the LL genotype in 82(19.1%). According to the functional classification, the 0L group was the most frequent (n=204, 47.4%), followed by the 1L group (n=182, 42.3%), while the 2L group was the least common (n=44, 10.2%) shown in Table-II. Pathan group had the highest frequency of the L allele (47.3%) while Sindhi group had lowest (39.4%). LA allele was common in Pathans (36.5%) and least common in Sindhis (30.8%). Therefore, difference between in allele distribution and ethnic group was statistically significant as *p*-value=0.012 shown in Table-III. At baseline, the mean HAMD scores were similar among the LL, LS and SS genotype groups 23.1±4.2, 23.4±4.6, and 23.4±5.0, respectively. After 12 weeks of treatment, HAMD scores decreased in all three groups, indicating improvement in depressive symptoms. LL genotype showed the greatest improvement, with the mean HAMD score decreasing to 9.2±3.8 representing a 60.1% reeducation from baseline. LS genotype also showed improvement with a Week 12 mean HAMD score of 10.8±4.5 and 53.8% reduction. SS genotype showed the least improvement, with a Week 12 mean HAMD score of 16.1±5.2 and 31.2% reduction. The differences in treatment response among the three genotype groups were statistically significant as *p*-value<0.001 shown in Table-IV.

DISCUSSION

This study provides the first comprehensive report of SLC6A4 polymorphism frequencies in a multi-ethnic Pakistani population. Our findings reveal that Pakistan has a unique genetic architecture with S allele predominance (0.574), placing it between European (S=0.40-0.43) and East Asian (S=0.70-0.80) populations[15]. This intermediate position aligns with Pakistan's geographical location and genetic history as a crossroads between South Asia, Central Asia, and the Middle East[16]. Pakistani allele frequencies differed significantly from European populations (L=0.57-0.60, χ^2=24.3, p<0.001) and East Asian populations (L=0.20-0.30, χ^2=11.8, p=0.003)[15], but were similar to South Indian populations (L=0.41, χ^2=0.85, p=0.36).[11]

Table-I: Demographic data of the study Participants (n=430)

Variables		Values
Age in Years	Mean± SD	32.29 ± 6.8
Gender	Male	210(48.8%)
	Female	220 (51.2%)
Ethnicities	Punjabi	189 (43.9%)
	Sindhi	99 (23.0%)
	Pathan	74 (17.2%)
	Balochi	48 (11.2%)
	Kashmiri/Northern Areas	20 (4.7%)
Educational status	Uneducated/ Unlettered	5 (1.2%)
	Basic/ under matriculation	163 (37.9%)
	Secondary Education	198 (46.0%)
	Higher Secondary Education	48 (11.2%)
	Graduates	12 (2.8%)
	Post-Graduates	4 (0.9%)

Table-II: SLC6A4 allele and genotype frequencies in Pakistani Cohort (n=430)

Classification of	Frequency
Alleles	
L allele	366 (42.6%)
S allele	494 (57.4%)
LA allele	279 (32.4%)
LG allele	87 (10.2%)
Genotypes	
LL	82 (19.1%)
LS	202 (47.0%)
SS	146 (33.9%)
Functional Groups	
0L	204 (47.4%)
1L	182 (42.3%)
2L	44 (10.2%)

Table-III: SLC6A4 allele Frequencies by Pakistani Ethnic Group (n=430)

Ethnic Group	Allele frequencies n (%)				*p*-value
	L	S	LA	LG	0.012
Punjabi	166(43.9)	212(56.1)	126(33.3)	40(10.6)	
Sindhi	78 (39.4)	120(60.6)	61 (30.8)	17 (8.6)	
Pathan	70 (47.3)	78 (52.7)	54 (36.5)	16 (10.8)	
Baloch	39 (40.6)	57 (59.4)	30 (31.3)	9 (9.4)	
Kashmiri	17 (42.5)	23 (57.5)	13 (32.5)	4 (10.0)	
Overall	366(42.6)	494(57.4)	284 (33.0)	86 (10.0)	

Table–IV: Comparison of Baseline and Week 12 HAMD Scores According to SLC6A4 Genotype (n=430)

Genotype	Baseline HAMD Score (Mean±SD)	Week 12 HAMD Score (Mean±SD)	Mean Reduction (%)	*p*-value
LL	23.1±4.2	9.2±3.8	60.1	< 0.001
LS	23.4±4.6	10.8±4.5	53.8	
SS	23.4±5.0	16.1±5.2	31.2	

The L allele frequency of 0.426 is similar to reports from South India (L=0.41)[18], reflecting shared genetic ancestry within the South Asian gene pool. However, the significant inter-ethnic variation within Pakistan (p=0.012) underscores the importance of considering ethnic diversity even within national boundaries. Pathans, with ancestral links to Central Asia, showed the highest L allele frequency (0.473), while Sindhis, with stronger South Asian genetic influences, showed the lowest (0.394). These differences may contribute to observed variations in antidepressant response across ethnic groups within Pakistan. The tri-allelic classification provides clinically relevant information. The LA allele frequency of 0.324 indicates that approximately one-third of alleles confer high serotonin transporter expression, while the combined S+LG frequency of 0.676 suggests that two-thirds confer low expression.[12] In clinical terms, this means that many Pakistanis may have genetically determined lower baseline serotonin transporter availability. Since SSRIs work by blocking these transporters, individuals with lower baseline expression might experience more pronounced serotonergic effects with treatment, potentially leading to both faster initial response but also greater adverse effects[19]. Neuroimaging studies have confirmed that S allele carriers show lower serotonin transporter binding potential in various brain regions.[13]

The functional group distribution has important implications for antidepressant prescribing in Pakistan. Nearly half the population (47.4%) falls in the 0L group (low expression), who theoretically would benefit from different treatment approaches than the 10.2% in the 2L group (high expression). Previous pharmacogenetic studies have shown that 0L group patients typically show poorer response to SSRIs and higher adverse effect burden compared to 2L group patients.[14] The association between S alleles and increased adverse effects during SSRI treatment has been documented in multiple studies.[15]

Our findings align with global meta-analyses showing ethnic variations in SLC6A4-antidepressant response associations. However, the effect sizes observed in our accompanying clinical data (manuscript in preparation) appear stronger than those reported in European populations, possibly due to differences in allele frequencies and genetic background.[16]

These findings have several implications for clinical practice in Pakistan. First, the high frequency of low-expression genotypes suggests that alternative first-line antidepressants (e.g., SNRIs, atypical antidepressants) might be more appropriate for many Pakistani patients than SSRIs. Second, for patients prescribed SSRIs, those with low-expression genotypes might require lower starting doses to minimize adverse effects. Third, in resource-constrained settings, selective pharmacogenetic testing for patients with previous treatment failure could be cost-effective. Previous research has demonstrated that pharmacogenetic testing can improve antidepressant treatment outcomes.[17] The significant differences between Pakistani and other global populations highlight limitations in generalizing pharmacogenetic findings across ethnic groups. Most commercial pharmacogenetic tests are developed and validated in European populations and may require recalibration for Pakistani patients. Similarly, antidepressant clinical trials conducted predominantly in European or East Asian populations may not accurately predict response patterns in Pakistan.[18]

Future research should establish normative allele frequencies through population-based studies, investigate gene-environment interactions in the Pakistani context, examine other relevant pharmacogenes (CYP2C19, CYP2D6) in combination with SLC6A4, and develop pharmacogenetic algorithms specifically for Pakistani populations. The role of SLC6A4 in antidepressant treatment response continues to be an active area of research, with recent studies emphasizing the importance of considering multiple genetic variants simultaneously.[19,20]

LIMITATIONS OF STUDY

This study has limitations. While we sampled all major ethnic groups, some groups (particularly Kashmiri) had modest sample sizes. We focused on a clinical population with MDD; population-based studies would provide more generalizable allele frequencies. We did not assess other SLC6A4 polymorphisms that might influence function, nor did we examine potential gene-gene interactions with other pharmacogenes.

CONCLUSION

The Pakistani population shows a unique distribution of SLC6A4 polymorphisms with S allele predominance

(0.574) and substantial inter-ethnic variation. The high frequency of low-expression genotypes (47.4%) suggests that many Pakistanis may be genetically predisposed to variable responses to SSRIs. These population-specific data are crucial for developing pharmacogenetic strategies tailored to the Pakistani population and for optimizing antidepressant therapy in clinical practice. As precision medicine advances in Pakistan, incorporating population genetics into treatment decisions will be essential for improving mental health outcomes.

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Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

MFA & SAZ: Data acquisition, critical review, approval of the final version to be published.

RR & KF: Conception, study design, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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